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ERRATA AND AUTHORS' EMENDATIONS

Page 67, first figure in column 3 of Table 1, "0.051" should be "0.51."

Page 286, fifth line from bottom, "usages" should be "usage."

Page 287, line 3, delete the words "present usage."

Page 316, line 7 in first paragraph under "Experimental Results," "seed produced," should be "produced seed."

Page 318, fifth and sixth lines from bottom should be "Cowpeas. *Vigna catjang sinensis:* Early Buff. Whippoorwill from Georgia."

Page 408, line 12 in second paragraph under "Emergence in Screen Cages," "Table 2, A–G, and Figure 7, A–G" should be "Table 2, A–E, and Figure 7, A–E."

Page 503, in list of plants, delete the word "Chinese" under heading "Common name" opposite *Pyrus amygdaliformis, P. balansae, P. canescens, P. cotinifolia, P. paschia,* and *P. sinaica.*

Page 503, in footnote *a* the word "oriental" should be "foreign."

Page 514, legend for Figure 7, "1 with Hiley" should be "2 with Hiley."

Page 600, line 8 in second paragraph, "Willis and Carrero (*15*)" should be "Gile and Carrero (*2*)." In following paragraph, line 12, "Willis and Carrero" should be "Gile and Carrero."

Page 617, Figure 9, B is upside down, and the letters placed on the wrong tubers. Transpose "*a*" and "*b*."

Page 636, Figure 3. Neither the actual nor the theoretical curve should go as high as shown. The actual curve should be broader and flatter at the apex, reaching its peak at ordinate 165.5 and abscissa 263, then passing to abscissa 264 at ordinate 180.5, then to abscissa 263 at ordinate 185.5 from which point it descends as shown. The theoretical curve should go only as high as abscissa 270.

Page 637, the formula just above Figure 4 should be a part of footnote 3 on page 636.

Page 645, the sentence beginning in line 11 should read: "Considering the error of three replications 1/40-acre plots as 2.837 per cent, the expected error, instead of 1.959 which was actually found (Table 5), three times the difference between two replicated series is 12 per cent."

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INHERITANCE OF RESISTANCE IN OATS TO PUCCINIA GRAMINIS AVENAE [1]

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INTRODUCTION

The production of disease-resistant plants by hybridization has engaged the attention of biologists with increasing interest since 1878, when Darwin (*6*)[3] reported the production by James Torbitt of a fungus-proof potato. It was not until 20 years later, however, that Farrer (*10*) demonstrated the production of rust-resistant cereals. Varietal resistance, hybridization for the production of new resistant varieties, and the factorial analysis of the inheritance of resistance have been extensively studied during the last decade.

Henning (*15*), in Sweden, and later, Hungerford and Owens (*16*), in this country, have shown that there is a marked difference in the susceptibility of wheat varieties to *Puccinia glumarum* (Schm.) Erikss. and Henn. Hungerford and Owens, in greenhouse and field tests, showed many of the common wheats to be resistant. In 1920 Melchers and Parker (*20*) reported three Crimean hard winter wheats that were resistant to leaf rust, *P. triticina* Erikss.

Mains and Leighty (*18*), working with rye, an open-pollinated plant, found that 68 different selections were resistant to *Puccinia dispersa* Erikss.

The reaction of oat varieties to *Puccinia coronata* Corda and *P. graminis* Pers. has been studied by Parker (*23*). Of the 120 strains tested, 80 were susceptible to both rusts. White Tartar and Ruakura Rustproof proved resistant to *P. graminis*, while Burt and several others of the red-oat group (*Avena byzantina* C. Koch) were resistant to *P. coronata*. In 1920 Durrell and Parker (*8*) made a comprehensive survey, involving the assembling of data for five years, on the response of oat varieties to crown and stem rusts under field conditions. White Russian and Green Russian were found to possess a marked resistance to *P. graminis*. In a previous report (*7*) by the present writer, Richland (Iowa No. 105) was shown to be resistant to stem rust.

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[1] Received for publication Apr. 17, 1928; issued September, 1928. The investigations here recorded were conducted by the Office of Cereal Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture, in cooperation with the Botany and Plant Pathology Section of the Iowa Agricultural Experiment Station. This article and the one entitled "The Alternate Hosts of Crown Rust, *Puccinia coronata* Corda," in the Journal of Agricultural Research 33: 953-970, 1926, were submitted by the writer to the graduate faculty of the Iowa State College in partial fulfillment of the requirements for the degree of doctor of philosophy.

[2] The writer wishes to express his thanks to I. E. Melhus, plant pathologist of the Iowa Agricultural Experiment Station, and to C. R. Ball and H. B. Humphrey, of the Office of Cereal Crops and Diseases, for suggestions and criticisms during the progress of the work and the preparation of the manuscript; also to the many assistants who ably aided in the collection of the data presented.

[3] Reference is made by number (italic) to "Literature cited," p. 22.

Although the varietal response to the rusts is known, it often is necessary to produce new varieties by hybridization. Biffen (*3, 4, 5*) found susceptibility to *Puccinia glumarum* dominant and secured a monohybrid segregation in the F_2 generation. On the other hand, Nilsson-Ehle (*22*) concluded that distinct dominance of susceptibility to this rust was of rare occurrence. Hayes, Parker, and Kurtzweil (*14*), in their investigation of varietal resistance of wheat to *P. graminis tritici* Erikss. and Henn., found the F_2 and F_3 generations segregating for resistance and susceptibility, although sterility probably prevented an expression of the true ratios. Parker (*24*) showed that resistance to *P. coronata* was heritable and suggested a multiple factor explanation. In 1922, Garber (*11*) found resistance in oats to stem rust, *P. graminis avenae* Erikss. and Henn., to be dominant and due to a single-factor difference in Minota-White Russian and Victory-White Russian crosses.

Stakman and Levine (*26*), Melchers and Parker (*19*), and others have shown the existence of 37 physiologic forms of rust within *Puccinia graminis tritici*, and Stakman, Levine, and Bailey (*27*) later isolated four such forms within *P. graminis avenae*. Because these specialized forms exist, it is imperative to determine the response of wheat and oat varieties to each of them. Puttick (*25*), Aamodt (*1*), Harrington and Aamodt (*12*), and Hayes and Aamodt (*13*) have studied the reaction of wheat hybrids to many of these forms.

The possible existence of linkage between rust resistance and other characters has been shown by Hayes, Parker, and Kurtzweil (*14*) and by Waldron (*28*) in wheat, and by Garber (*11*) in oats. In none of these cases was the linkage sufficiently close, however, to prevent the production of varieties which were rust resistant and endowed with other desirable characters.

The manner of inheritance of rust resistance in wheat has been demonstrated by Hayes, Parker, and Kurtzweil (*14*) to be different for a durum-common and an emmer-common cross. Whether such a difference can be obtained in oat species and varieties has not previously been known. It is the purpose of this paper to report the manner of inheritance of resistance to *Puccinia graminis avenae* by eight varieties of oats belonging either to *Avena sativa* L. or *A. byzantina* C. Koch.

METHODS AND MATERIALS

Preceding a consideration of the inheritance of rust resistance in oats, adaptations of existing methods and the development of many new ones were necessary. The fact that many data were taken on each individual plant necessitated detailed planting, harvesting, and recording methods, together with special means of producing an epidemic of the rust and estimating its subsequent effect on these plants.

SOURCE OF OAT VARIETIES

The eight pure-line varieties of oats used as parent material of the hybrids treated in this study are described below.

The strain of Burt was a head selection from C. I. No. 710 in 1916. It is a reddish black oat belonging to the species *Avena byzantina* and is susceptible to *Puccinia graminis avenae* but moderately resistant to *P. coronata*.

The Early Ripe variety was a head selection from the material obtained by J. H. Parker, formerly of the United States Department of Agriculture, from H. H. Love, of Cornell University, Ithaca, N. Y. It is similar to Burt except that it has a finer straw and usually a lighter colored grain. It has fewer basal hairs and a less pronounced cavity at the base of the first floret. It is susceptible to both oat rusts.

The Green Russian variety was a selection from Minnesota No. 350 and belongs to the species *Avena sativa*. It is resistant to stem rust and moderately resistant to crown rust.

The Richland (Iowa No. 105) variety is a yellow selection from Kherson produced by L. C. Burnett, of the Iowa Agricultural Experiment Station, in cooperation with the United States Department of Agriculture. The material used here was a head selection from this pure line. It is an extremely early oat, an excellent yielder, and resistant to *Puccinia graminis avenae*. It often escapes *P. coronata* under field conditions in Iowa because of its early maturity.

The Lincoln variety was obtained from H. H. Love, of Cornell University. It is similar to Swedish Select, except that two-kerneled spikelets predominate. It yields well in the cooler sections of the United States, especially New York, but is susceptible to stem rust.

National, a variety similar to Silvermine, also was obtained from Cornell University. It is a good yielder, of mid-season maturity, but susceptible both to *Puccinia graminis avenae* and *P. coronata*.

The Ruakura variety was obtained from a head selection made in 1916 from C. I. No. 701. It is a slender-stemmed, early-maturing oat belonging to the species *Avena byzantina*. Ruakura is similar to Burt in vegetative growth, but has pubescent nodes. It possesses a moderate degree of resistance to both oat rusts.

According to Etheridge (*9*), the White Russian and White Tartar varieties are indistinguishable. White Russian was a head selection from Minnesota No. 5, whereas White Tartar was obtained from Cornell University. Both of these are side-panicled, late-maturing, low-yielding strains, belonging to *Avena sativa* var. *orientalis*. They possess marked resistance to *Puccinia graminis avenae*.

SOWING

The seeds were sown in 10-foot rows spaced 1 foot apart. In 1920, the individual seeds were spaced 6 inches apart in the row, but this distance was reduced to 4 inches in all the subsequent years of the experiment. In both the F_1 and F_2 sowings, only the primary kernel was used, the second floret or "pin" oat having been removed. This method permitted only one plant to emerge in a single space, thereby removing the difficulty of separating heavily tillered plants.

The F_1 seeds were divided into three lots. The first portion was sown in 1920 and produced the F_2 generation. F_2 plants were grown from the second portion in 1921 adjacent to the F_3 generation and compared with it. The third portion, with a similar remnant of F_2 seed and some of the original crossed seed, was sown in 1922, so that the F_1, F_2, and F_3 generations were grown side by side in that year.

Two series of control rows were necessary. Every tenth row was sown to the same susceptible pure-line variety to gauge the uniformity of the epidemic produced in the nursery, and the resistant or susceptible pure-line parents were sown every twenty-fourth and twenty-

fifth row, respectively, in order to compare their rust response with that of their progeny. The culture of the nursery was uniform.

RECORDING

Each cross was given a number. For instance, White Russian×Burt was given number 274, which was used for all subsequent generations. Each F_1 plant of this cross or its reciprocal was given a subnumber; that is, 274–1, 274–2, 274–3, etc. Reciprocal crosses were made and their progeny studied to determine the differential influence, if any, on the progeny. No differences were noted, however, and in order to simplify the presentation of the data a cross and its reciprocal are considered as a single cross.

Each plant was given a position number. This method allowed for comparison of the response of each individual plant with each adjoining plant in the office records as well as under field conditions. The following data were taken on each plant: Dates of seeding, heading, maturing, and harvesting; percentage of rust infection; size of uredinia; height; shape of panicle; and yield.

HARVESTING

All of the individuals of the F_1 and F_2 generations were harvested separately. The panicles of each plant were wrapped in a separate paper and the product of the entire row was inserted in a paper bag. Each F_3 plant was labeled with a string tag, and the plants in the entire row were bagged together but threshed as individual plants.

TECHNIC OF PRODUCING EPIDEMICS

The classification of the individual plants into either the resistant or the susceptible group depended upon their relative response to inoculation by *Puccinia graminis avenae*. Theoretically, this classification was based upon the fact that all plants had equal opportunity for maximum infection. In order to afford this opportunity, the following methods of exposing the plants were used:

(1) Oat plants infected with stem rust were transplanted directly into the nursery from the greenhouse. These plants had been infected with urediniospores collected from the nursery in the previous year and maintained in greenhouse stock cultures.

(2) Urediniospores were scraped from oat plants in the greenhouse, placed in distilled water, and sprayed on the culms with an atomizer.

(3) Urediniospores were scraped from oat plants in the greenhouse and dusted on the previously moistened oat plants in the field.

(4) Urediniospores were applied directly to the leaves of the oat plants by means of a scalpel between 7 and 8 o'clock p. m. The plants thus exposed were covered with a bell jar for 12 hours.

In 1919 and 1920 plants in the field were exposed to infection when 6 to 9 inches high. It was noted, however, that very few became visibly infected until the panicles had just emerged from the sheath. From 1921 to 1923, therefore, the plants were not exposed until this stage of maturity was reached.

CLASSIFYING PLANTS BY SIZE OF UREDINIA

Each individual plant was classed as resistant or susceptible by using the scale devised by N. A. Cobb in Australia and later revised and used by the Office of Cereal Crops and Diseases, United States

Department of Agriculture.[5] This scale is based on the proportion of the total area covered by uredinia, no consideration being given to the relative size of the sori, though it will be shown later that the size of the rust sorus can be correlated directly with resistance. In addition to the quantitative estimate of rust, the size of uredinia is used in this paper in classifying the response of the individual plants to rust.

Rust estimates were started on the standing plants about three weeks before harvest and continued until harvest. During the last week of this period the plants were harvested and estimated at the same time. Only one rust estimate was made for each plant. The whole plant was inspected and the maximum infection accepted as indicating its degree of resistance. It was not uncommon to find certain culms of a single plant more heavily infected than others. Uredinia and telia were estimated collectively during the last few days of each season, for the fungus rapidly enters the telial stage as the oat plant matures. Progenies of all plants which could not be classified as either susceptible or resistant under field conditions were tested in the greenhouse or in the field the following spring.

As noted above, the size of the uredinia was employed as a measure of susceptibility. (Fig. 1.) During the progress of this work the resistant pure-line varieties of oats developed a consistently low percentage of infection. Many of the susceptible varieties varied in reaction from apparent resistance to susceptibility. These results could be explained in one of two ways: (1) The pure-line varieties of oats were not homozygous for susceptibility to rust; or (2) they were homozygous and certain plants were escaping infection or at least severe infection. It then became necessary to find some other index by means of which these escaping plants might be classified according to their inherent response to rust. Such an index was found in the size of uredinia.

If, then, the size of uredinia can form the basis for differentiating susceptible and resistant plants, how are small and large uredinia to be distinguished? Although these two classes were only relative, they none the less were sharply defined. Hence the classification was reasonably accurate because the greater dimension of the large uredinia was more than ten times that of those classified as small.

Pure-line resistant varieties of oats, under field conditions, consistently showed a low percentage of infection and small-sized uredinia. Pure-line susceptible varieties differed greatly in percentage of infection, but uniformly produced large uredinia. In progeny tests of 100 individuals, having a low percentage of infection but large uredinia under field conditions, 1,649 plants were produced the next year with a high percentage of infection and large uredinia. Clearly, then, these plants had partially escaped stem rust the first year but could have been classified as susceptible on the basis of size of uredinia.

Although pure lines showed a positive correlation between size of uredinia and response of the host to rust, it was not known whether hybrids acted in the same way. In addition to the data on size of uredinia on F_1 plants recorded in Table 1, records of F_2 and F_3 plants of Green Russian × Richland and White Russian × Burt, and F_4

[5] Scale for estimating rust. In Cereal disease field notebook. U. S. Dept. Agr., Bur. Plant Indus., Cereal Invest., C. I. form 11. [June, 1915.]

plants of White Tartar × National and White Tartar × Lincoln were made. The susceptible F_1 plants, subsequently classified as susceptible by the reaction of both the F_2 and F_3 progeny, had large uredinia. An F_3 progeny test includes the F_3 individuals from a single F_2 plant. The F_1 individuals classified in Table 1 as resistant, and subsequently proving to be so, had small uredinia. In the total F_2 population in all years, 10,004 plants were classified as resistant and had small

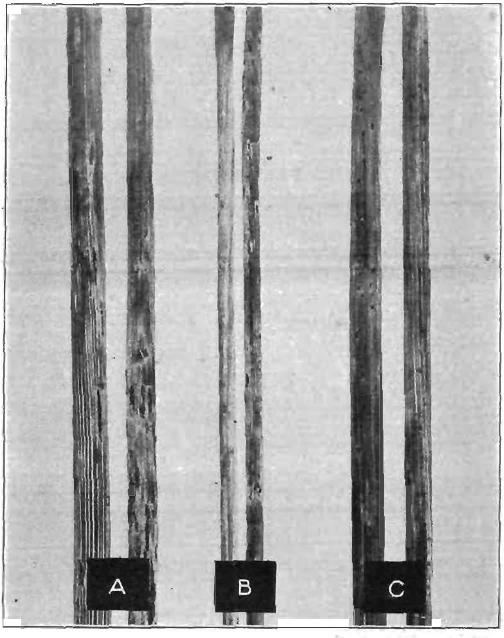

FIG. 1.—Size of urediniosori in an F_3 progeny of White Tartar × Lincoln: A, Large urediniosori (susceptible plant); B, intermediate urediniosori (susceptible plant); and C, small urediniosori (resistant)

uredinia, while 713 were susceptible, with large uredinia. In the test of the F_3 progeny, those rows segregating for rust reaction also segregated for size of uredinia, the individual susceptible plants having large uredinia.

Among the 2,700 F_2 plants studied for size of uredinia in 1920, all those placed in the large-uredinia group showed susceptibility in further progeny tests.

TABLE 1.—*Reaction of F_1 plants to Puccinia graminis avenae, showing correlation between size of uredinia and rust susceptibility*

Cross	Hybrid No.	Number of seeds set	F_1 reaction to P. graminis avenae (number of plants)			
			Resistant		Susceptible	
			Size of uredinia		Size of uredinia	
			Large	Small	Large	Small
White Russian×Burt	274	27	0	15	12	0
Green Russian×Burt	283	10	0	2	8	0
Siberian×Burt	1206	6	0	0	6	0
White Russian×Ruakura	271	2	0	0	2	0
Green Russian×Early Ripe	280	2	0	2	0	0
Green Russian×Richland	277	5	0	5	0	0
Total		52	0	24	28	0

It should be pointed out that not all plants having small uredinia are resistant, but that any of these plants, grown under field conditions, could be classed as susceptible if large uredinia were present on the culms, regardless of the percentage of infection.

Time of maturity perhaps is one of the chief causes for lack of perfect correlation between size of uredinia and resistance. All of the 100 rust-escaping plants mentioned above were mature from 7 to 10 days earlier than their susceptible sisters. It is probable, then, that maturity, accompanied by senility of the host cells, prevented further development of this obligate parasite. This conclusion is supported by the fact that many of the susceptible early-maturing plants had both large and small uredinia, indicating that the uredinia develop but little after the maturity of the host. Progeny from these plants, when infected at an earlier stage in the next year, were susceptible and produced only large uredinia.

The correlation of size of uredinia and rust reaction under greenhouse conditions is not so marked as in the field. As all stem-rust inoculations were made on the leaf blades in the greenhouse and on the culms in the field, some difference would be expected. Type of infection indicated by hypersensitive areas assisted in differentiating the oat plants grown in the greenhouse into either resistant or susceptible groups.

HYBRIDIZING IN THE GENUS AVENA

Before the production of oat hybrids was begun in 1919, a survey of the literature was made, but this revealed little information on the technic of crossing oat varieties under field conditions. Although field crossing can be done very successfully in some sections of the United States, in other sections successful crosses are obtained with difficulty owing probably to such factors as temperature and humidity. In the opinion of the writer, field crossing should be practiced only when greenhouse facilities are not available, as oats sown in the greenhouse in November, and heading in March, permit a high percentage of successful crosses. A consideration of such problems as length of time between emasculation and pollination, time of day best suited for

pollinating, and quantity of pollen necessarily precedes the successful production of oat hybrids.

PERIOD BETWEEN EMASCULATION AND POLLINATION

In making an oat cross, the anthers must be removed from the inclosing lemma and palea before the pollen is shed on the stigmatic surface. It would be more convenient to remove the anthers and immediately insert the foreign pollen. Using White Russian as the female parent and Burt as the male, pollinations were made at the time of emasculation and at the end of each succeeding 12-hour interval up to 72 hours under field conditions. The first series, consisting of 10 pollinations on each of two panicles, was made at 7.30 o'clock a. m., the next at 7.30 o'clock p. m. The general method of pollination for all hybrids reported below involved cutting all except 10 spikelets from the panicle of the female parent on emergence from the sheath. The second floret was removed from each of these spikelets and the remaining primary florets were emasculated. An oil-paper bag was then placed over each prepared panicle and tied at the base with a string.

The results showed about the same percentage of seeds produced from pollinations at each of these periods. However, in testing these seeds, the F_1 plants indicated that 6 out of 20 of the resulting seeds were self-fertilized when emasculation and pollination occurred at the same time. A higher percentage of hybrids was produced when pollination occurred 48 hours after emasculation.

EFFECT OF TIME OF DAY ON SEED SETTING

Using the White Russian × Burt cross, the influence of time of day on set of seed was determined under field conditions. Pollinations were made at Ames, Iowa, and Iron River, Wis., at intervals from 4.30 o'clock a. m. until 8.30 o'clock p. m. on three consecutive days at each place.

At these two places 770 pollinations were made on 77 different panicles. The results are presented in Table 2. From these pollinations 78 seeds resulted, an average of 10.1 per cent for the entire experiment. From the forenoon pollinations 36 seeds were obtained and 42 from those made in the afternoon. During the six days no seed was set from pollinations made between 11.30 a. m. and 2.30 p. m. The failure to set seed on June 28 at Ames is difficult to explain, as the mean temperature and general characteristics were similar on these three days except for low relative humidity on June 28.

At Ames, out of a total of 180 pollinations made in the forenoon 17 were successful, as compared with 9 from a total of 210 pollinations made in the afternoon. At Ames no seed was set from pollinations made between 10.30 a. m. and 3.30 p. m.

At Iron River, Wis., 19 out of a total of 180 attempts were successful in the morning and 33 from a total of 210 in the afternoon. The interval during which no seeds were set was about two hours shorter at Iron River than at Ames. It is probable that such factors as temperature and relative humidity influence the effectiveness

TABLE 2.—*Influence of time of day on effectiveness of cross-pollinating White Russian and Burt oat varieties under field conditions*

Place and date in 1919	Number of seeds resulting from cross-pollination at time of day indicated												
	Forenoon							Afternoon					
	4.30	5.30	8.30	9.30	10.30	11.30	12.30	2.30	3.30	4.30	5.30	7.30	8.30
Ames, Iowa:													
June 26	4	2	1	2	0	0	0	0	0	0	1	1	-----
June 27	2	2	4	0	0	0	0	0	0	2	1	3	1
June 28	0	0	0	0	0	0	0	0	0	0	0	0	0
Iron River, Wis.:													
July 29	2	3	1	0	0	0	0	0	0	1	1	1	2
July 30	1	1	1	0	0	0	0	0	0	3	2	2	3
July 31	2	1	1	3	3	0	0	0	4	3	4	3	4
Total	11	9	8	5	3	0	0	0	4	9	9	10	10

QUANTITY OF POLLEN

Although Jelinek (*17*) draws no conclusions concerning the quantity of pollen necessary to fecundation, this may be a factor in the explanation of his results in the production of wheat hybrids by the following methods: (1) Insertion of a whole anther in the female floret, and (2) emasculation of spikes and bagging them together with spikes of similar maturity belonging to the pollen parent. He states that in 1916 the second method resulted in twice as much seed production as the first. In the unfavorable year of 1917 no seed was produced by the first method, whereas 50 per cent of the florets on 24 out of 47 spikes produced seed by the second method.

An easy method of pollination is to insert a whole anther from the male parent between the palea and lemma of a floret of the female parent. However, when using this method in studies here recorded, fewer seeds developed than when a small quantity of pollen was dusted on the stigmatic surface, even though all pollinations were made early in the morning and at least 24 hours after emasculation. Upon examining the florets of crosses made by inserting the whole anther between the lemma and palea, many molds were found growing abundantly on the surface of the caryopsis. The excess pollen probably served as a favorable medium for these molds and their action often prevented the formation of viable seed.

PHYSIOLOGIC FORMS OF RUST IN THE NURSERY

According to Stakman, Levine, and Bailey (*27*), four distinct physiologic forms of stem rust occur on oats, varying widely in their varietal reaction. Only two of these were found in America. As certain varieties of oats are resistant to one form and susceptible to another, it is imperative in any study of inheritance of rust resistance that the class of reaction to the fungus be known. The results obtained by Stakman and his associates naturally raise the question as to what form was prevalent in the nursery from year to year. Unfortunately, the geographic range of the physiologic forms found in America has not yet been determined. As no effective method, other than the one employed, has been devised to prevent physiologic forms from infecting oat hybrids grown under field conditions, it was

necessary to determine which form or forms were present each year. Four methods were used to accomplish this.

In the fall of 1919, a composite sample of *Puccinia graminis avenae* was taken to the greenhouse and maintained in stock culture in the urediniospore stage (*21*). This culture of rust was used to start the initial field infection in the spring of 1920. In the fall a composite sample of rust again was taken from the nursery and used as inoculum on nine pure-line parents. If these parents responded in the same manner to the 1919 and 1920 cultures, the 1919 culture was discarded and the later culture used in the field the next spring. This process was continued throughout the study.

As an additional test for the physiologic response of *Puccinia graminis avenae*, 100 pure-line varieties of oats were grown adjoining the oat-breeding nursery each year, every tenth row being the same pure-line control. These indicated the uniformity of the epidemic.

As already described, the initial infection was started each spring in this nursery with the same culture of *Puccinia graminis avenae* that had been overwintered in the greenhouse. During this five-year period, all varieties showing resistance in 1919 were resistant in the following four years and those susceptible in 1919 were uniformly susceptible thereafter. Moreover, each pure-line parent sown in the breeding nursery showed similar results each year from 1919 to 1923. The results thus far indicate either that only one physiologic form was present in the nursery during the entire time or that these pure lines did not act as differential hosts.

To identify the physiologic form employed in this investigation, the differential hosts of Stakman, Levine, and Bailey (*27*) were exposed to infection. A composite sample of stem rust from the oat-breeding nursery was taken to the greenhouse in July, 1923, and White Tartar, Monarch (C. I. 1760), and the awnless Monarch Selection of Etheridge (C. I. 1879) were inoculated. Monarch Selection of Etheridge (C. I. 1879) was susceptible and White Tartar resistant. According to Stakman and others (*27*), Monarch Selection (C. I. 1879) reacts as a differential host for forms 1 and 2, being resistant to the former and susceptible to the latter. These results suggest that only form 2 (*27*) was present, as Monarch Selection bore only one type of infection, which was normal, with numerous large, coalesced uredinia.[6]

HYBRID VIGOR OF F_1 PLANTS

In crossing certain varieties of oats, remarkable hybrid vigor, expressed as yield and height, was shown by the F_1 plants. (Fig. 2.) Some of these F_1 plants produced more than 2,200 seeds and varied in yield from 10.5 to 36 gm. The parents of these crosses were grown in the same year in field rows adjacent to the F_1 hybrids, but were greatly inferior in yielding capacity. In another cross, Richland (Iowa No. 105), the highest yielding parent, produced only about one-fifth as much as the lowest yielding hybrid of which it and Green Russian were the parents. It should be mentioned that oats make excellent material for genetic studies, as sufficient numbers can be readily obtained in the F_1 generation to make an F_2 inheritance study significant.

[6] Since the completion of these investigations, it has been shown by Bailey (*2*) that only certain selections of Monarch Selection of Etheridge act as differential hosts for physiologic forms 1 and 2 of *Puccinia graminis avenae*.

The yield in grams and the height in centimeters are the averages of at least 20 parental plants grown under the same cultural conditions as the hybrids. As shown in Table 3, the height in the F_1 did not

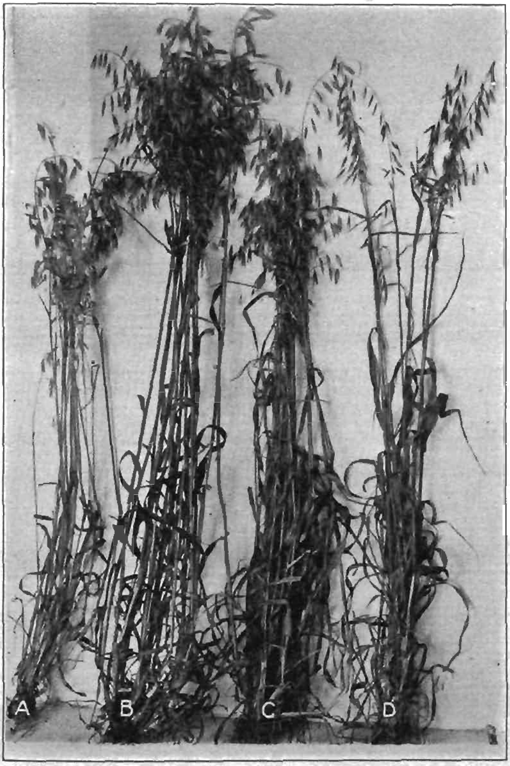

FIG. 2.—Hybrid vigor expressed in height in a White Russian × Burt cross. A, Burt parent; B, F_1 plant; C, F_2 plant; D, White Russian parent

increase in proportion to the yield, but usually was intermediate between that of the parents. Reciprocal crosses had a response similar to those reported in Table 3.

TABLE 3.—*Vigor of parent and F_1 plants of oats in 1919, as expressed by yield and height*

Parents and hybrids	Hybrid No.	Number of plants	Average yield (grams)	Average height (cm.)
White Russian		20	2.7	98
Ruakura		20	1.5	63
White Russian×Ruakura	271	2	22.5	89
Burt		20	2.1	70
White Russian		20	2.7	98
White Russian×Burt	274	4	14.1	90
Richland		20	3.8	79
Green Russian		20	2.6	97
Green Russian×Richland	277	5	21.5	100
Early Ripe		20	1.4	70
Green Russian		20	2.6	97
Green Russian×Early Ripe	280	2	27.5	94
Burt		20	2.1	70
Green Russian		20	2.6	97
Green Russian×Burt	283	3	19.7	91

FACTORIAL EXPLANATION OF RUST RESISTANCE IN OATS

Although rust resistance of cereals has been known to be a heritable character since 1898, when Farrer (*10*) produced resistant wheats by hybridization, the manner of inheritance was not clearly understood until lately. In order to predict the results of a cross between resistant and susceptible oat varieties, the manner of inheritance must be determined. A study of the segregation of the F_2 into resistant or susceptible plants, as verified by the behavior in the F_3, affords the basis for a factorial explanation. An attempt is made to explain the following crosses on a factorial basis.

CROSSES OF RESISTANT AND SUSCEPTIBLE VARIETIES

The first step in the usual method of obtaining material for a factorial explanation of the inheritance of any character involves crossing two individuals differing sharply with respect to this character. After determining the relative reaction of oat varieties to *Puccinia graminis avenae*, crosses were made between resistant and susceptible varieties. Obviously, more can be learned by making the other possible crosses, namely, resistant on resistant and susceptible on susceptible varieties. In this study, hybrids were produced only by crossing resistant with susceptible and resistant with resistant varieties.

RUST REACTION OF THE F_2 PLANTS

If resistance to rust is dominant, all individuals in the F_1 of the cross between resistant and susceptible varieties should be resistant. However, in the present experiments both resistant and susceptible F_1 plants were obtained from crosses involving the susceptible Burt and certain resistant varieties. As it has been shown that all of the 1,115 Burt plants examined were susceptible, this variety must be homozygous for susceptibility to stem rust.

In hybrids obtained from Green Russian×Early Ripe (hybrid No. 280–1 in Table 4), the F_1 was resistant and the F_2 segregated into 254 resistant and 64 susceptible plants. This approximates a 3:1 ratio. In a reciprocal cross (280–2) involving the same parents, the F_2 segregated into 250 resistant to 102 susceptible plants, again approaching a 3:1 ratio. Resistance is dominant and the cross can be explained by the assumption of a single-factor difference. Green

Russian could be symbolized as *SS* (resistant) and Early Ripe as *ss* (susceptible). The F_1, being *Ss*, would be resistant, and the F_2 would segregate in the proportion of 1*SS* : 2*Ss* : 1*ss*, or a 3:1 ratio.

TABLE 4.—*Rust reaction of F_1 and F_2 plants from crosses between resistant and susceptible varieties*

Cross	Hybrid No.	F_1		F_2		Ratio		Probable error	$\frac{D}{PE}$
		Resistant (S)	Susceptible (s)	Resistant (S)	Susceptible (s)	Calculated	S:s		
Green Russian × Early Ripe	280-1	S	....	254	64	238.5:79.5	3:1	±5.21	2.97
Early Ripe × Green Russian	280-2	S	....	250	102	264:88	3:1	±5.48	2.55
Green Russian × Burt	283-2	....	s	30	111	26.4:114.6	3:13	±3.13	1.15
Do	283-3	S	....	179	54	174.8:58.2	3:1	±4.46	.94
White Russian × Burt	274-4	....	s	23	77	25:75	1:3	±2.92	.68
Do	274-5	S	....	185	45	174:56	3:1	±4.43	2.48
Do	274-6	....	s	58	251	57.9:251.1	3:13	±4.63	.02
Do	274-8	S	....	26	8	25.5:8.5	3:1	±1.70	.29

White Russian × Burt (hybrid No. 274-6, Table 4) was susceptible in the F_1 and in the F_2 segregated into 58 resistant and 251 susceptible plants, thus closely approximating a 3:13 ratio. This cross may be explained on the basis of a two-factor difference, one of which was a resistance inhibitor.

The genotypic composition of the Burt parent in this case can be considered as *ssII*, when *s* = susceptibility and *I* = a resistance inhibitor. The genotype of White Russian, the resistant parent, would be *SSii* where *S* = resistance and *i* = absence of resistance inhibitor. With such an assumption, the F_1 would be *SsIi* or susceptible. The F_2 would segregate in the ratio of 3 resistant to 13 susceptible plants.

The actual results gave a close approximation to the theoretical, *PE* being ±4.63 and $\frac{D}{PE}$ equaling 0.02.[7] The F_3 results from this cross will be considered in detail in Table 5.

It is apparent from the above hypothesis that three genetically different strains of Burt might be obtained, each of which would breed true for susceptibility and maintain the three genetic compositions, namely, *SSII*, *ssII*, and *ssii*. The fact that these three different genotypes do exist, and breed true for susceptibility in what was thought to be a pure line of Burt, will be shown later. Hybrid No. 274-4, White Russian × Burt (Table 4), was susceptible in the F_1 and segregated into 23 resistant to 77 susceptible plants in the F_2. Fitting this cross to a 1:3 ratio resulted in a *PE* of ±2.92 and $\frac{D}{PE}$ of 0.68. The actual and the theoretical results do not entirely agree, but the deviation probably is not significant. This cross thus can be explained by assuming *SSii* as the genetic composition of White Russian and *SSII* as that of Burt.

In two other crosses of White Russian × Burt (hybrid Nos. 274-5 and 274-8, Table 4), the F_1 plants were resistant. No. 274-5 segregated into 185 resistant and 45 susceptible plants in the F_2. This approximates a 3:1 ratio, the *PE* of which was ±4.43 and $\frac{D}{PE}$

[7] *PE* = probable error; $\frac{D}{PE} = \frac{\text{deviation}}{\text{probable error}}$.

equaled 2.48. The F_2 of No. 274–8 segregated into 26 resistant and 8 susceptible plants. Assuming a 3:1 ratio, the PE was ± 1.70 and the $\frac{D}{PE}$ 0.29. Both of these could then be satisfactorily explained by assuming *SSii* as the factorial composition of White Russian and *ssii* as that of Burt.

The F_1 derived from a Green Russian × Burt cross was resistant (hybrid No. 283–3, Table 4). The F_2 contained 179 resistant and 54 susceptible plants. Explaining this cross on the basis of a 3 : 1 ratio, the PE was ± 4.46 and the $\frac{D}{PE}$ 0.94. Another F_1 plant (hybrid No. 283–2) was susceptible, and the F_2 segregated in the ratio of 30 susceptible to 111 resistant plants. Interpreting this result as representing a 3 : 13 ratio, the PE was ± 3.13 and the $\frac{D}{PE}$ was 1.15. Green Russian could then be represented as *SSii* and Burt as *ssii*.

It should be pointed out here that Green Russian and White Russian crosses can be explained by assuming the same genetic composition for both of these pure-line parents, while Burt, which breeds true for susceptibility to rust, has at least three different genetic compositions which breed true for susceptibility.

Rust Reaction of the F_3 and F_4 Plants

WHITE RUSSIAN×BURT

As shown in Table 4, one cross of White Russian × Burt (hybrid No. 274–6) was susceptible to *Puccinia graminis avenae* in the F_1 generation. The segregation following this cross differed from that of many other crosses made both by Garber (*11*) and by the writer in that the F_2 generation contained 3 resistant to 13 susceptible plants. These F_2 resistant plants showed, on an average, in the F_3 progeny test that one bred true for resistance, while two progenies split in the ratio of 3 resistant to 1 susceptible plant. (Fig. 3.)

Placing these results on a factorial basis, the following factors were assumed: S = resistance, s = susceptibility, I = resistance inhibitor, i = absence of inhibitor.

White Russian, then, might be represented as *SSii* and Burt as *ssII*. The following outline would then represent the reaction of the parents and the F_1, F_2, and F_3 generations:

White Russian *SSii* (resistant) × Burt *ssII* (susceptible)		
F_1 reaction	F_2 individual plant reaction	F_3 progeny tests
SsIi susceptible.	1 *SSII* susceptible	Homozygous susceptible.
	2 *SSIi* susceptible	1 resistant, 3 susceptible.
	2 *SsII* susceptible	Homozygous susceptible.
	4 *SsIi* susceptible	3 resistant, 13 susceptible.
	1 *SSii* resistant	Homozygous resistant.
	2 *Ssii* resistant	3 resistant, 1 susceptible.
	1 *ssII* susceptible	Homozygous susceptible.
	2 *ssIi* susceptible	Do.
	1 *ssii* susceptible	Do.
	16	

This hypothesis allows a satisfactory explanation of the actual results obtained in this cross of White Russian×Burt (hybrid No. 274–6) as shown in Table 5.

It is well to point out in this connection that the criterion of the whole behavior in this cross is the susceptibility of the F_1 and the two

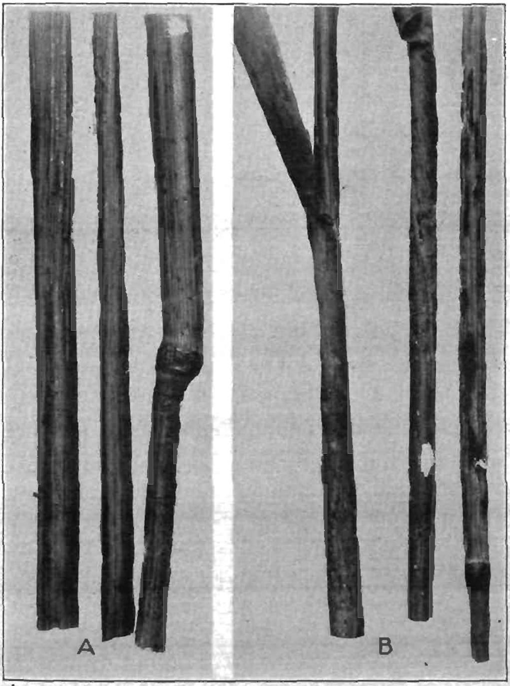

FIG. 3.—Culms from two F_1 plants of a White Russian×Burt cross. These two plants grew side by side in the same nursery row: A, susceptible; B, resistant

genetically distinct resistant F_2 hybrids, produced in the proportion of one which breeds true for resistance to two which are heterozygous and segregate into three resistant plants to one susceptible plant. A summary of the breeding behavior in the F_3 is presented in Table 5.

TABLE 5.—*Breeding behavior for rust reaction of F_3 families of oats grown from seed of individual F_2 plants of crosses between White Russian and Burt (274–6)*

Breeding behavior in the F_2	Breeding behavior in the F_3									
	Number of homozygous resistant		Number of heterozygous with ratio of—						Number of homozygous susceptible	
			3:1			1:3 and 3:13				
				Plants			Plants			
	Families	Plants	*Families*	Resistant	Susceptible	*Families*	Resistant	Susceptible	Families	Plants
58 resistant: Observed	19.0	348	39.0	723	229					
58 resistant: Calculated	19.3	348	38.7	714	238					
58 resistant: Deviation	.3		.3	9	9					
251 susceptible: Observed						111.0	748	2,869	140.0	4,755
251 susceptible: Calculated						115.9	753	2,864	135.1	4,755
251 susceptible: Deviation						4.9	5	5	4.9	

The 58 resistant F_2 plants produced 19 F_3 families homozygous for resistance where the expectation was 19.3, and 39 heterozygous where the expectation was 38.7. These latter segregated in the ratio of three resistant to one susceptible plant. The deviation of 0.3 needs no further consideration. The 251 susceptible F_2 plants produced 140 F_3 families homozygous for susceptibility and 111 heterozygous where the expectation was 135.1:115.9. Insufficient plants of the F_3 families were grown to differentiate the 3:13 and 1:3 ratios, respectively.

WHITE TARTAR×NATIONAL

Through the kindness of H. H. Love, of Cornell University, 86 F_2 plants of White Tartar × National (278a) were sent to the writer at Ames, Iowa, in the spring of 1921. Seed from part of these plants was sown that spring and seed from the remaining part in 1923. The National variety is extremely susceptible to stem rust, and White Tartar is resistant. (Fig. 4.) The F_1 and F_2 generations of this cross had been grown at Ithaca, N. Y., where their reactions to stem rust were not recorded. From these 86 F_2 plants, 2,100 F_3 individuals were produced. Of these F_3 families 20 bred true for resistance, 19 bred true for susceptibility, and 47 segregated, producing 854 resistant to 303 susceptible plants. The data for both 1921 and 1923 are summarized in Table 6.

Placing this cross on a factorial basis, White Tartar could be expressed as *SSii* and National as *ssii*. A ratio of 3 resistant to 1 susceptible would be expected in the F_2. In the F_3, 1 F_2 plant should breed true for resistance, 2 should segregate in a ratio of 3 resistant plants to 1 susceptible plant, and 1 should breed true for susceptibility. Explaining this cross on a 3 : 1 ratio, it is found that the resistant F_2 had a PE of ± 2.70 and the $\frac{D}{PE}$ was 0.9. The 47 heterozygous progenies splitting into 854 resistant to 303 susceptible plants had a PE of ± 9.93 and the $\frac{D}{PE}$ was 1.38.

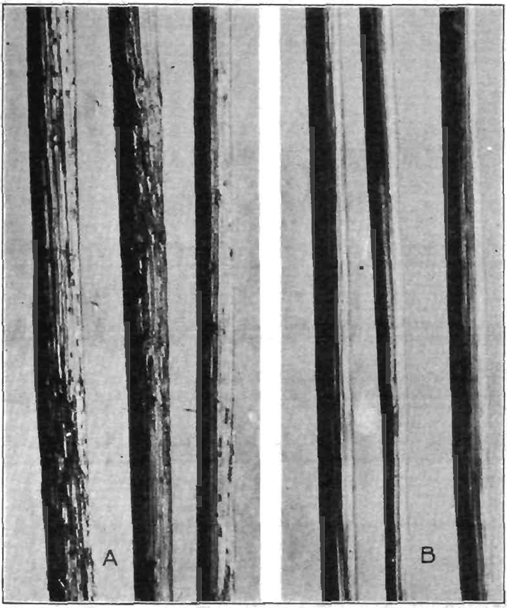

FIG. 4.—Characteristic appearance of susceptible and resistant parents in an oat cross: A, National (susceptible); B, White Tartar (resistant)

TABLE 6.—*Breeding behavior for rust reaction of 86 F_3 families of oats grown from seed of individual F_2 plants of crosses between White Tartar and National (278a)*

Ratio	Breeding behavior in the F_3						
	Number of homozygous resistant		Number of heterozygous			Number of homozygous resistant	
	Families	Plants	Families	Plants: Resistant	Plants: Susceptible	Families	Plants
Observed	20.0	478	47	854	303	19.0	465
Calculated	21.5	478	43	868	289	21.5	465
Deviation	1.5	----------	4	14	14	2.5	----------

Assuming a 3:1 ratio, the 86 F_2 plants should have segregated in a ratio of 21.5 homozygous resistant to 43 heterozygous to 21.5 homozygous susceptible families. (Table 6.) The deviation of the calculated from the observed data needs no further comment.

Progenies of 83 plants of the F_3 were studied in the F_4 generation. All plants selected from homozygous resistant and homozygous susceptible F_3 rows bred true for resistance or susceptibility, respectively. Both resistant and susceptible F_3 plants were selected from the heterozygous F_3 rows and their progeny carried through the F_4 generation. These susceptible F_3 plants bred true for susceptibility, whereas two-thirds of the resistant plants segregated in a ratio of three resistant plants to one susceptible, and the other third bred true for resistance.

WHITE TARTAR×LINCOLN

The 175 F_2 plants of the cross White Tartar×Lincoln (253a) were sent to Ames from Ithaca and have the same history as the White Tartar×National. Lincoln is susceptible to stem rust while White Tartar is resistant. (Fig. 5.) From these F_2 individuals 4,405 plants were grown in the F_3. Of the F_3 families, 38, containing 948 plants, were homozygous for susceptibility; 44, containing 1,094 plants, were homozygous for rust resistance; 93, containing 2,363 plants, segregated in a ratio of 1,828 resistant plants to 535 susceptible plants.

Placing these results on a factorial basis, White Tartar could be expressed as *SSii* and Lincoln as *ssii*, where S = resistant and s = susceptible. A ratio of 3 resistant plants to 1 susceptible would be expected in the F_2. Assuming a 3:1 ratio, the deviation between the calculated and observed data is small. (Table 7.)

TABLE 7.—*Breeding behavior for rust reaction of 175 F_3 families of oats grown from seed of individual F_2 plants of crosses between White Tartar and Lincoln (253a)*

Ratio	Breeding behavior in the F_3						
	Number of homozygous resistant		Number of heterozygous			Number of homozygous susceptible	
			Families	Plants			
	Families	Plants		Resistant	Susceptible	Families	Plants
Observed	44.0	1,094	93.0	1,828	535	38.0	948
Calculated	43.7	1,094	86.5	1,773	590	43.7	948
Deviation	.3		6.5	55	55	5.7	

In 1922, 101 F_3 plants were studied for their reaction to rust in the F_4 generation. Of these 101 plants, 26, selected from a homozygous resistant F_3 progeny produced 342 F_4 plants, all of which were resistant. Eight susceptible F_3 plants, selected from homozygous susceptible F_3 progenies, bred true for susceptibility in the F_4 generation by producing 107 susceptible plants to none that was resistant.

Theoretically, there should have been three different kinds of plants in the heterozygous F_3, namely, two kinds of resistant and one susceptible. From the segregating F_3 families 43 resistant plants

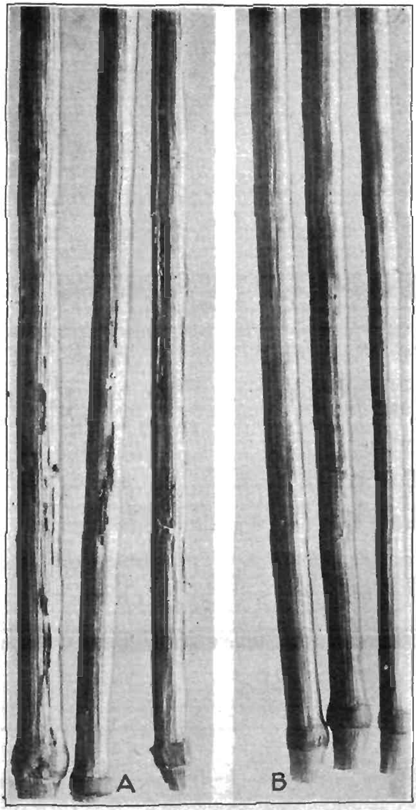

FIG. 5.—Characteristic appearance of susceptible and resistant parents in an oat cross: A, Lincoln (susceptible); B, White Tartar (resistant)

were selected. Of this number, 12 bred true for resistance, producing 177 plants, and 31 segregated in the ratio of 313 resistant to 111 susceptible. The PE here, assuming the 3:1 ratio characteristic of a monohybrid, is ±6.01 and the $\frac{D}{PE}$ is 0.831. Of the susceptible plants selected from the F_3, 24 bred true for susceptibility in the F_4, producing 291 plants. These F_4 families conform to expectation.

CROSSES OF RESISTANT VARIETIES

RUST REACTION OF THE F_2 AND F_3 PLANTS

In order to study further the inheritance of resistance to stem rust, crosses were made between the two resistant varieties, Green Russian and Richland. As shown earlier (7), Richland possesses inherent resistance to stem rust. Additional crosses were made between White Russian and Ruakura. The latter parent was resistant, but less so than White Russian.

GREEN RUSSIAN×RICHLAND

The F_1 plants of the cross between Green Russian and Richland were more resistant than either parent. In fact, only a few small rust pustules were found. The F_2 plants, as shown in Table 8, segregated with many resistant plants and only a few susceptible ones. Here, again, the resistant plants were more resistant than either parent. The susceptible plants were severely attacked. Hybrid No. 277-4 produced 309 resistant plants to no susceptible ones in 1922, but the remnant seed of this cross produced 186 resistant and 3 susceptible plants in 1923.

Another cross of Green Russian×Richland (hybrid No. 277-3) was studied in the F_3 in 1922 and in 1923. The one susceptible F_2 plant produced 13 susceptible plants in 1922 and 99 susceptible plants in 1923. The 1,000 F_4 plants obtained from the 1922 F_3 susceptible plants all bred true for susceptibility in 1923. In 1922, the progenies of 333 resistant F_2 plants segregated into 244 which bred true for resistance to 89 which segregated. As only 20 seeds from each F_2 plant were sown for the F_3 population, it is probable that 244 resistant plants to 89 susceptible do not express the true ratio.

TABLE 8.—*Reaction to Puccinia graminis avenae of the F_1 and the F_2 plants from crosses between two resistant varieties of oats, Green Russian and Richland*

Hybrid No.	F_1 reaction	Number and reaction of F_2 plants	
		Resistant	Susceptible
277-1	Resistant	322	0
277-2	do	346	0
277-3	do	265	1
277-4 (1922)	do	309	0
277-4 (1923)	do	186	3
277-5	do	256	0

The possibility of a mechanical mixture as an explanation of the behavior of this cross is eliminated by the fact that distinct segrega-

tion for height of plant as well as color of the lemma occurred. In addition, the resistant hybrids possessed a more marked resistance than either parent, and the susceptible hybrids were intermediate between the two parents in height of plant.

WHITE RUSSIAN×RUAKURA

Both White Russian and Ruakura were classified as resistant, but the latter consistently showed more stem rust. All of the F_1 plants of the four families carried through the F_2 were resistant, as shown in Table 9. The crosses of these parents responded similarly to those of Green Russian × Richland.

TABLE 9.—*Reaction to Puccinia graminis avenae of the F_1 and F_2 plants from crosses between two resistant varieties of oats, White Russian and Ruakura*

Hybrid No.	F_1 reaction	Number and reaction of F_2 plants	
		Resistant	Susceptible
271-1	Resistant	245	1
271-2	do	100	0
271-3	do	335	5
271-4	do	154	0
Total		834	6

A total of 834 resistant to 6 susceptible plants was produced by all four families. Without doubt the White Russian × Ruakura crosses were hybrids, as evidenced by the typical monohybrid segregation for shape of panicle in the F_2 generation and also by the segregation for height of plant.

No attempt was made to give a factorial explanation of the results obtained by crossing resistant on resistant varieties, as it is probable that insufficient numbers were obtained to express the true ratios in the F_3. It is probable, however, that factors in addition to those considered in this paper are involved in the inheritance of rust resistance in the Green Russian × Richland and the White Russian × Ruakura crosses.

SUMMARY

Out of 770 pollinations, no hybrids were obtained from pollinations made between 11.30 o'clock a. m. and 2.30 o'clock p. m. under field conditions in central Iowa and northern Wisconsin. Pollination at the time of emasculation resulted in some selfed seeds, whereas pollination 24 to 72 hours after emasculation produced true hybrids and a percentage of fertility higher than that resulting after longer periods. Dusting the stigma with pollen produced a percentage of hybrids higher than by inserting the whole anther between the lemma and palea.

Marked hybrid vigor as expressed by yield was obtained in the F_1 generation of oat crosses.

Large uredinia were directly correlated with susceptibility to *Puccinia graminis avenae*.

Resistance to *P. graminis avenae* is dominant and due to a single-factor difference in White Tartar × National and White Tartar × Lincoln crosses.

At least three genetically different strains of Burt were found to breed true for susceptibility. One of these carried a factor which was an inhibitor of resistance. In a Burt × White Russian cross, the F_1 generation was susceptible, the F_2 segregated in the ratio of 3 resistant to 13 susceptible plants. In the F_3, these resistant F_2 plants produced 1 homozygous resistant progeny to 2 progenies which segregated in the proportion of 3 resistant plants to 1 susceptible. In another White Russian × Burt cross the F_1 generation was resistant and the F_2 generation segregated in the ratio of 3 resistant plants to 1 susceptible plant. In still another White Russian × Burt cross, the F_1 generation was susceptible and the F_2 segregated in the proportion of 1 resistant to 3 susceptible plants.

In crosses between resistant varieties such as Green Russian × Richland and White Russian × Ruakura, the F_1 plants were resistant. The F_2 segregated and produced some plants which were more resistant than either parent. It is probable that other factors than the two considered here are involved in the inheritance of resistance to *Puccinia graminis avenae*.

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THE OCCURRENCE OF NOSE SPOTS AND TAIL SPOTS IN GUINEA PIGS[1]

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SCOPE OF PAPER

In the stock of guinea pigs maintained by the Bureau of Animal Industry for experimental purposes, it has often been observed among piebald animals that the nose is one of the portions most likely to be white. Many piebald animals have been recorded, however, which have the nose partly or entirely pigmented. The extent of the pigmented region varies from a small triangular area covering the tip of the nose and extending up to a point between the eyes to an area covering one side of the head and extending over the mid line, or even covering the whole head. It has been observed that this peculiar pattern occurs more frequently in one of the inbred families (family 2) than in the others. In 1918 a selective experiment was started to determine whether the nose-spot pattern could be fixed or increased. Nose-spot animals from family 2 were mated in an experiment called 2N. The present paper deals with the results of this experiment and of the various inbred and crossbred lines in which the nose-spot character occurs.

OCCURRENCE OF NOSE SPOT

Studies were made of the five existing inbred families and of the crossbreeding and control experiments to determine, if possible, the nature and inheritance of the pattern. As is shown in Table 1, the average occurrence of nose-spot animals in four of the inbred families (13, 32, 35, and 39) is 1 per cent, while family 2 shows 9.2 per cent. The two lines of family 2 also differ widely in the percentage of nose-spot animals which they produce; line 2–8–4,[2] which has produced 43.7 per cent of the young born in family 2, produced 14.4 per cent of nose-spot animals, while line 2–9–7, which has produced 36.4 per cent of the young born in this family produced but 3.3 per cent of nose-spot animals. The earlier lines of family 2, producing 18.1 per cent of the animals of this family, produced 9.6 per cent nose spot, practically the same as the average for the whole family. The selection experiment 2N has produced 14.2 per cent of nose-spot animals, which is practically equal to the number produced by the high-producing line of family 2. The crossbreeding experiments, those designated by C–O, CC, BX, etc., show considerable variation in the percentage of nose spot produced, but a study of these variations shows them to be of no statistical significance.

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[2] The symbols 2-8-4 designate the family, generation, and mating to which any animal belongs or from which it has descended. Thus, the above combination means inbred family 2, eighth generation of brother-sister mating, and mating No. 4 of this generation.

TABLE.—*Total number of animals and number and percentage of nose-spot animals in various lines of guinea pigs*

Stock	Total number of animals	Number of nose-spot animals	Per cent of nose-spot animals
Family 2	4,148	383	9.23
Family 13	5,118	34	[a] .66
Family 32	3,693	39	[a] 1.06
Family 35	3,803	46	[a] 1.21
Family 39	1,078	29	[a] 1.47
B	5,697	53	.93
C-O	1,304	41	3.14
C-1	108	7	6.48
C-2	35	2	5.71
CC	78	5	6.41
CY-O	193	5	2.59
CY-1	501	6	1.20
CY-2	171	6	3.51
BX	481	8	1.66
AC	437	14	3.20
CA	328	11	3.35
2O and 2L	38	5	13.16
Early lines of family 2	752	72	9.57
Line 2-8-4	1,812	261	14.40
Line 2-9-7	1,510	50	3.31
2N	648	92	14.20

[a] The average of families 13, 32, 35, and 39 is 1.014.

DIVISION OF FAMILY 2 INTO LINES [3]

In the development of any family from a common ancestor, different lines will gradually be formed due to differences in death rate, rate of reproduction, rate of mating, and other causes. Some lines will become stronger than others and persist, while the weaker ones will become extinct. One line may possess certain hereditary traits differing from another, so that after a few generations it becomes difficult to recognize any relationship between the two lines in question. This has taken place in family 2. A genealogical diagram of this family (fig. 1) shows that throughout its history new branches frequently appeared. Some of these soon became extinct, while others gave rise to numerous other branches, some of which persist to the present day. In this way, two large lines of family 2 have been formed, designated as lines 2-8-4 and 2-9-7. These trace to a common subline, 2-6-8. In the data dealing with the sublines of family 2, 2-9-13 has been included with Group 2-8-4, for the grandparents of 2-9-13 are the parents of 2-8-4. Group 2-9-4 has been included in Group 2-9-7, for both have the same parentage. Male 1 line is slightly different from the other lines of the family in that the foundation sire was mated to two of his daughters. This line was not carried far and only 74 animals were produced.

The early lines of family 2, including all lines up through those starting at the eighth generation except 2-8-4, produced a total of 752 animals, of which 72 had nose spots, giving a percentage of 9.6 and agreeing closely with the family average, 9.2 per cent. At this point a permanent separation occurred. Mating 2-8-4 gave rise to a large group which has produced 1,812 individuals with 261 nose spots

[3] Studies of each of the five inbred families now in existence have been made in detail and it has been found that each family is divided into lines, some quite noticeably different in various respects from others. These differences were especially noticeable in the nose spot and tail spot of family 2, otocephaly in family 13, and polydactyly in family 35.

among them, or a percentage of 14.4. Likewise, mating 2–9–7 gave rise to another large group producing 1,510 individuals with but 50 nose spots, a percentage of 3.3, which is far below the average for the whole family. If the several small sublines which make up the large groups are examined, considerable variation in the percentage of nose-spot animals produced will be noted and it will be seen that sublines of Group 2–8–4 are consistently much higher than the sublines in Group 2–9–7, with the exception of subline 2–9–4, which produced 19.4 per cent of nose spots. Had this line persisted it might have given rise to a group producing more nose spots even than line 2–8–4. A similar rise in percentage of nose-spot animals produced appears to be taking place in sublines 2–15–6, with 21.7 per cent nose spots, and in 2–18–1, with 26.5 per cent. All lines

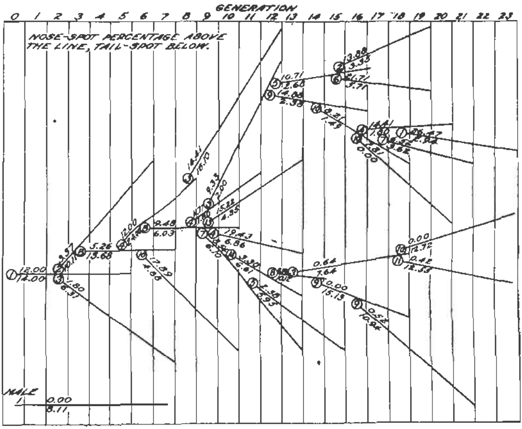

FIG. 1.—Lines of family 2 and percentage of nose-spot and tail-spot animals in each

tracing to mating 2–9–7 have been consistently low producers of nose-spot animals, some rather large sublines not having produced a single nose spot.

It is interesting to note that there is also a difference in these two large groups of family 2 in respects other than nose spot. In detailed studies of the inbred families of guinea pigs a difference in the amount of pigment in the coat of some lines was found. Group 2–8–4 showed a low percentage of white—55.4 per cent for males and 68.9 per cent for females—while 2–9–7 showed 82.7 per cent for males and 87.7 per cent for females. This difference can not be accounted for by the presence of the nose spot. In arranging the normal and nose-spot animals in the two lines of family 2 and experiment 2N according to the distribution of white, one sees that there is very little difference between the nose-spot animals and those without the nose spot

within the same line, the difference being for the 2-8-4 line about 2.5 per cent, for the 2-9-7 line and for 2N about 5 per cent. Males show a slightly larger difference than females. When all groups are considered together, the males show a difference of about 4.5 per cent white between animals with nose spot and those without it; and females a difference of about 2.8 per cent. These differences and the range of white are shown graphically in Figure 2.

These differences can be accounted for by the nose spot, as will be seen in an explanation of the grading system used. At birth, a

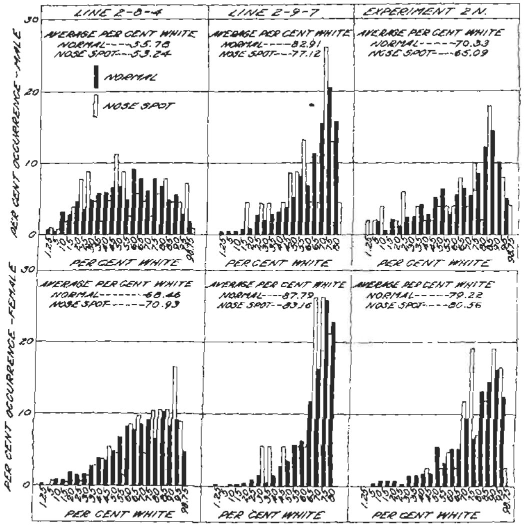

FIG. 2.—Percentage of white and frequency of occurrence of normal and nose-spot animals in family 2 and experiment 2N

rubber-stamp outline of the dorsal and ventral surfaces of the guinea pig is made and filled in by vertical or horizontal lines to represent the extent of differently colored areas, black being shown by vertical lines, and red by horizontal lines. A transparent pattern of exactly the same outline is divided into 10 areas of equal size for the dorsal pattern and 8 for the ventral; the feet and legs are counted as 2 divisions, thus making the whole surface of the animal divided into 20 areas, each representing 5 per cent of the area of the animal. The percentage of color of each animal is determined by laying the transparent divided pattern over the diagram of the animal in question, and recording the number of parts of color on the dorsal and ventral

surfaces separately. These parts can readily be converted into percentages by multiplying by five. In general, the pigmented area on the nose occupies one of these divisions, equaling about 5 per cent of color; thus a nose-spotted animal would show approximately 5 per cent less of white than an animal with an unpigmented nose.

The size of the litter is slightly smaller in line 2–9–7 (2.39 ± 0.026) than in 2–8–4 (2.41 ± 0.023), the difference being 0.02 ± 0.035. Along with differences in litter size there is also a difference in weight at birth and at weaning, these being for the 2–8–4 line, 72.283 ± 0.277 and 179.066 ± 0.810 gm., respectively, and for the 2–9–7 line 74.732 ± 0.317 and 180.441 ± 0.946 gm., respectively. The differences between the two lines are slight, 2.449 ± 0.421 gms. for birth-weight difference and 1.375 ± 0.246 gm. for weaning weight. By using a correction factor to get the weights all on the basis of an average size of litter, the average litter size being regarded as 2.5, the birth weight for the two lines under consideration becomes 71.56 gm. for the 2–8–4 and 73.76 gm. for the 2–9–7 line. Weaning weights are 177.63 gm. and 178.64 gm., respectively. The difference still persists and is but slightly reduced by the correction factor.

These several differences between two large groups of family 2 indicate some distinct differences in their genetic constitution which are transmitted from generation to generation, and in the case of nose spotting seems to give rise to higher-producing lines at certain points in the history of the family. This condition agrees with the results found in family 13 as regards the production of otocephaly (*7*).[4] Throughout the history of this family a deformity known as otocephaly has appeared. One and fifty-four hundredths per cent of the animals born were of the otocephalic type. At various points in the history of the family a rise in the percentage of otocephaly occurred, and finally, in the thirteenth generation, two distinct lines separated, one producing 2 per cent of otocephali, the other 8.9 per cent. Even in this high-producing line certain sublines produced as high as 21.5 per cent of otocephalic young, suggesting the possibility of still higher producing lines in future generations.

RESULTS OF CROSSING

The first cross between the various inbred families has been designated as C–O. Only crosses in which the present five families are involved have been included in the data of this paper. C–1 is composed of brother-sister matings from C–O, and C–2 of brother-sister matings from C–1, so that both involve only two inbred families. CC is a cross between two C–O matings, and therefore usually involves four inbred families. BX is a back cross, a C–O mated back to one of the parental inbred lines. AC and CA involve three inbred families, the C parent being from a C–O mating and the A parent being an animal from one of the inbred families. The CY experiment differs from the C experiment in that only family 2 and a certain line of family 13 are involved. 2G and 2L are selection experiments from family 2, but only a few animals are involved in these experiments. (See Table 1.)

Some of the different experiments are comparable, then, and Table 2 groups them according to their similarity, and whether the family 2

[4] Reference is made by number (italic) to "Literature cited," p. 41

parent came from the high or low nose-spot-producing line. Since family 13 produced the fewest nose spots, crosses between this family and family 2 and the F_2 and F_3 young of these crosses have been grouped separately, for the purpose of studying segregation. While the data presented show that the high line of family 2 does produce more nose spot in crosses with family 13 than the low line, the differences are not statistically significant. This difference is in the reverse direction in crosses with the other inbred families. Comparisons between succeeding generations of the cross between families 2 and 13 show in general a rise of the percentage of nose spot in the F_2 and F_3 generations, but here again the differences are statistically not significant.

A tabulation of the various crosses with respect to the percentage of family 2 ancestry shows also very little difference in the percentage of nose spot produced. Animals which have one-quarter, one-half, or three-quarters of the blood of family 2 show very little difference in the percentage of nose spot produced.

TABLE 2.—*Number and percentage of nose-spot young in crosses of family 2 with other inbred families*

Family 2 crossed with—	Experiment	High nose-spot line of family 2: Total number of young	High: Number of nose-spot young	High: Per cent of nose-spot young	Low nose-spot line of family 2: Total number of young	Low: Number of nose-spot young	Low: Per cent of nose-spot young	Difference
Family 13	C-0	134	5	3.73	235	2	0.85	
	CY-0	120	3	2.50	73	2	2.74	
Total F_1		254	8	3.15± .739	308	4	1.30± .435	1.85±0.858
Family 13	C-1	8	0	0	37	4	10.81	
	CY-1	263	6	2.28	238	0	0	
Total F_2		271	6	2.21± .602	275	4	1.45± .486	.76± .774
Family 13	C-2				14	1	7.14	
	CY-2	104	5	4.81	67	1	1.49	
Total F_3		104	5	4.81±1.415	81	2	2.47±1.163	2.34±1.832
2×13 difference:								
Between F_1 and F_2				.94± .954			.15± .652	
Between F_1 and F_3				1.66±1.597			1.17±1.242	
Between F_2 and F_3				2.60±1.538			1.02±1.261	
Family 32	C-O	128	3	2.34	189	4	2.11	
Family 35	C-O	166	5	3.01	104	7	6.73	
Family 39	C-O	75	3	4.00	105	6	5.71	
Total of above, 3 crosses.		369	11	2.98± .597	398	17	4.27± .684	1.29± .908

PERCENTAGE OF NOSE-SPOT YOUNG CLASSIFIED BY FAMILY 2 ANCESTRY

Ancestry	Total number of young	Number of nose-spot young	Per cent of nose-spot young
One-fourth blood, family 2	1,286	35	2.722±0.300
One-half blood, family 2	2,312	67	2.898± .235
Three-fourths blood, family 2	38	3	7.895±2.950
Difference:			
Between one-fourth and one-half bloods, family 2			.176± .386
Between one-fourth and three-fourths bloods, family 2			5.173±2.966
Between one-half and three-fourths bloods, family 2			4.997±2.960

RESULT OF SELECTION FOR NOSE SPOT

In 1918 a selection experiment called 2N was started for further study of the nose-spot character. Animals from family 2 possessing a nose-spot were mated among themselves regardless of their relationships. The animals used were not separated as to generations, but any showing nose spot were mated. This experiment is still in progress. Through December, 1926, there had been 648 animals produced with 92 nose-spots, a percentage of 14.2 which is practically equal to the percentage in the nose-spot-producing lines of family 2. There is much similarity between 2N and family 2 in weight of individuals, size of litter, mortality, and amount of white. The percentage of males is somewhat lower in 2N than in family 2, being 46.42 and 50.37 per cent, respectively. Differences in these experiments are given in Table 3.

TABLE 3.—*Difference in various characters between the lines of family 2 and experiment 2N, and between nose-spot animals and litter mates without nose spot*

Line or experiment	Birth weight	Size of litter	Per cent born alive	Per cent raised of those born alive	Per cent of all young raised to weaning	Per cent white		Sex ratio (per cent males)
						Male	Female	
	Grams	*Number*						
2-9-4	72.283	2.41	80.545	83.399	67.174	55.37	68.89	51.269
2-9-7	74.732	2.39	83.279	83.918	69.896	82.72	87.66	52.306
Total family 2	74.565	2.39	81.768	84.257	68.895			50.374
Nose-spot animals from family 2	66.700	2.72	86.680	83.130	72.060	56.89	72.44	51.060
Litter mates not showing nose spots	66.050	2.68				60.54	75.55	
Total 2N	70.380	2.49	82.250	84.240	69.290	69.42	78.66	46.420
Nose-spot animals from experiment 2N	69.390	2.80	80.430	87.840	70.650	65.08	80.56	64.350
Litter mates not showing nose spots	68.280	2.81				71.75	82.85	

It is of interest to study 2N according to the ancestry of the parents as regards nose spot. This study can be made in two ways. One is by classifying the young as to whether one, or both, or neither parent was from the high-producing nose-spot line of family 2. By this method of classification it was found that when both parents were from the low-producing nose-spot line 10.9 per cent of the young had a colored nose; when one parent was from the high-producing line and the other from the low, 12.2 per cent had a nose spot, and when both parents were from the high-producing line 14.9 per cent had nose spot. (Table 4.) The numbers entering into these percentages are small, the remainder of the animals of this experiment having been produced by parents of mixed ancestry from those original matings directly from family 2. Although there is a gradual rise in the percentage of nose-spot animals produced, the differences between the groups are not significant.

The second way in which 2N may be studied is by classifying the young as to the number of nose-spot ancestors they had for three generations back, i. e., to the great-grandparents. It is seen that in cases where neither parent nor any of the grandparents or great-grandparents had nose spot, a smaller percentage of the young had nose spot; the average being slightly over 9 per cent when parents or

grandparents are considered, and nearly 7 per cent in the case of great-grandparents. When one or more of the parents, grandparents, or great-grandparents had a nose spot, a larger percentage of the young also had this character, but this increase is not consistent with the increase of nose-spot ancestors.

TABLE 4.—*Amount of nose spot in 2N classified by ancestral family 2 lines*

Ancestry	Total number of young	Number of nose-spot young	Per cent of nose spot young
Both parents from low nose-spot line of family 2	46	5	10.87±3.094
1 parent from high nose-spot line of family 2	82	10	12.20±2.4378
Both parents from high nose-spot line of family 2	67	10	14.92±2.9350
Difference:			
Between Groups 1 and 2			1.33±3.9401
Between Groups 1 and 3			4.05±4.2862
Between Groups 2 and 3			2.72±3.8160

AMOUNT OF NOSE SPOT IN 2N CLASSIFIED BY NUMBER OF NOSE-SPOT ANCESTORS

Relationship	Number of nose-spot ancestors								
	0	1	2	3	4	5	6	7	8
Parents:									
Total number of young	32	59	557						
Number of nose-spot young	3	12	77						
Per cent of nose-spot young	9.38	20.34	13.82						
Grandparents:									
Total number of young	53	87	105	114	289				
Number of nose-spot young	5	12	12	21	42				
Per cent of nose-spot *young*	*9.43*	*13.79*	*11.43*	*18.42*	*14.53*				
Great-grandparents:									
Total number of young	29	21	111	111	63	22	132	6	153
Number of nose-spot young	2	2	16	14	12	3	21	1	21
Per cent of nose-spot *young*	*6.90*	*9.52*	*14.41*	*12.61*	*19.05*	*13.64*	*15.91*	*16.67*	*13.72*

In family 2 and in experiment 2N a selective process has been going on, in the case of family 2 unconsciously, in 2N consciously. As family 2 was subdividing into lines, gradually the frequency with which nose-spot animals were produced increased in certain matings and in the lines arising from these matings. Thus, there gradually arose the one line producing over 14 per cent of nose-spot animals and the other line producing only a little more than 3 per cent. Since family 2 has been maintained wholly by brother-sister matings, all matings have been made at random so far as the nose-spot character is concerned. In this case the similarity between the mated animals has been primarily genetic and only incidentally somatic. In 2N the matings have been made on somatic resemblance incidentally genetically similar because of the several generations of close inbreeding behind them. This accounts for the great similarity in family 2 and 2N in respect to weight, mortality, and amount of white.

In discussing the effects of selection, Wright (*3*) says that random mating may be followed in a selected line without loss of progress

toward homozygosis. Selection of heterozygotes leads to no fixation of characters. After an indefinite number of generations of selection a return to random breeding brings the population again to an unselected condition. Almost the full effect of selection is reached in the first generation if the average of the parents is of the desired type, and further selection merely reduces the variability. The rate at which characters can be fixed is much increased by combining inbreeding and assorted mating based on somatic resemblance. These statements seem to explain why 2N does not show a higher percentage of nose spot than the nose-spot lines of family 2. In reference to genetic similarity, family 2 has been selected, but in relation to nose spot the matings were made at random. The whole family 2 population, therefore, represented an unselected condition, but one line of this family, through unconscious selection, was already of a certain desired type. Then, when further selection was made from this type for experiment 2N, no increase in the nose-spot character resulted. The rapidity with which 2N showed results comparable with the 2-8-4 line is due to the combined inbreeding and selection.

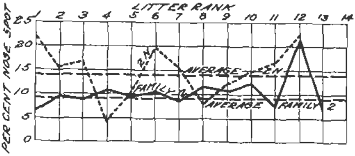

FIG. 3.—Percentage of nose spot by litter rank

RELATION OF NOSE SPOT TO OTHER CHARACTERS

LITTER RANK AND SEASON OF BIRTH

In other studies (*4*, *5*, *6*) of the guinea-pig experiments, certain relationships between different characters were found. A study of some of these characters has been made to determine whether there is any relationship between them and nose spotting. The first character studied was litter rank. Tabulations were made to determine the percentage of nose-spot guinea pigs produced in succeeding litters. A slight tendency for the percentage to rise in later litters of family 2 was noted. The percentage in 2N, however, fluctuated considerably, due to small numbers, but in general it followed close to the average line. This relationship is shown in Figure 3. The percentage of nose-spot young born in any month shows a close agreement with the average. Again, 2N shows much more variation than family 2. (Fig. 4.)

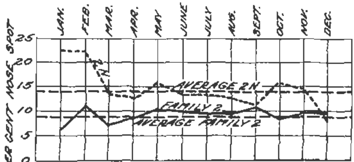

FIG. 4.—Month of birth of nose-spot guinea pigs

SIZE OF LITTER

In determining the size of litters in which nose-spot guinea pigs were born, the individual, rather than the litter, was taken as the unit, the litters being weighted, therefore, by the number of individuals. This gives a more satisfactory comparison where individuals of a litter are concerned than the litter as a whole. The averages thus obtained are somewhat higher than averages derived from using the litter as the unit.

In family 2 three divisions were made in comparing the size of litters in which nose-spot animals were born with the size of the litters in which their normal brothers and sisters were born. The first division included the early lines of the family before the separation into two distinct lines took place. Nose-spot animals were born in litters averaging 2.72 in size (Table 5), and their normal brothers and sisters in litters of 2.68. Line 2–8–4 nose-spot animals were produced in litters averaging 2.75, while their normal brothers and sisters were born in litters of 2.70. Line 2–9–7 nose-spot animals were produced in litters of 2.54 average size while their normal brothers and sisters were born in litters of 2.60. Family 2 as a whole produced nose-spots in litters of 2.72 average size and their normal brothers and sisters were born in litters of 2.68, a difference of 0.04 ± 0.034. In 2N, nose-spot animals were born in litters of 2.80 average size and their normal brothers and sisters in litters of 2.81. The differences and the probable errors show that nose-spot animals are not born in appreciably larger or smaller litters than their normal brothers and sisters.

TABLE 5.—*Litter and size of nose-spot guinea pigs and of their normal brothers and sisters*

Lines	Size of litter 1: Nose-spot animals	1: Normal animals	2: Nose-spot animals	2: Normal animals	3: Nose-spot animals	3: Normal animals	4: Nose-spot animals	4: Normal animals	5: Nose-spot animals	5: Normal animals	Total: Nose-spot animals	Total: Normal animals
Early lines, family 2	7	32	26	126	25	122	8	24	6	29	72	333
2–8–4	18	91	79	485	118	545	42	226	4	16	261	1,363
2–9–7	5	32	22	126	14	121	9	47	0	5	50	331
2N	5	27	28	180	41	232	16	104	2	13	92	556

Lines	Average size of litter: Nose-spot animals	Average size of litter: Normal animals	Difference
Early lines, family 2	2.72± 0.084	2.68± 0.038	0.046± 0.092
2–8–4	2.75± .036	2.70± .016	.051± .039
2–9–7	2.54± .086	2.60± .033	.058± .092
2N	2.80± .061	2.81± .025	.009± .066

Since the study of nose spot has involved animals almost wholly from family 2, it will be expected at once that there will be a close similarity to family 2 in weight. Since in other studies of weight in

guinea pigs (*4*, *5*), there has been found a high correlation between birth weight and weaning weight, only birth weight has been considered in this study. The average birth weight of all young in family 2 was 74.6 gm. The average birth weight of all nose-spot young in family 2 for all sizes of litters was 70.9 gm.; in 2N it was 69.4 gm. In comparing the weight of the nose-spot animals with the weight of their litter mates without nose spot a close agreement is found. In family 2 the weight of nose-spot animals which had litter mates was 66.7 gm., while the weight of their normal litter mates was 66 gm. In 2N, nose-spot animals weighed 69.4 gm. and their normal litter mates weighed 68.3 gm. The differences are small and not significant.

MORTALITY

The mortality of the guinea pigs used in the investigations of the Bureau of Animal Industry has been measured by three percentages: The percentage of young born alive, the percentage raised of those born alive, and the percentage of all young raised to weaning. These percentages for the two large lines of family 2 and 2N are shown in Table 3. It will be seen that there is a very close agreement in these percentages, indicating that there is little if any relation between nose spot and mortality.

SEX

Sex ratio in these experiments has been expressed as percentage of males. The percentage of males in family 2 as a whole is 50.4 ± 0.524. The percentage of males among the nose-spot young is 51.1 ± 1.734. Experiment 2N as a whole produced 46.4 ± 1.341 per cent males, while among the nose-spot young the percentage of males was 54.4 ± 3.503. These apparently wide departures in 2N are probably due to small numbers as compared with family 2. The difference is 7.93 ± 3.75, which can not be considered statistically significant.

COLOR OF NOSE SPOT AND COAT-COLOR RELATIONS

Family 2 is a tricolor—black, red, and white. In classifying the nose-spot individuals as to color it was found that there was a large excess of animals having red spots. In family 2, out of 383 animals which showed this pattern there were 111 with black nose and 272 with red nose, or 28.98 and 71.02 per cent, respectively. Experiment 2N produced 92 nose spots, 23 black, and 69 red in percentages of 25 and 75, respectively.

To determine whether this relation holds also for other parts of the body a tabulation was made of the color of the right shoulder and left hip. An equal area was observed on each animal by cutting out squares of equal area over shoulder and hip on a transparent pattern which was laid over the original color diagram of the animal. The color that filled the largest portion of this cut-out area was recorded as the color for that particular spot. There were 3,082 animals observed, of which 1,055 had either a black or red right shoulder. In the same group 1,027 had a colored hip.

A further study was made by tabulating the amount of black, red, and white in the two lines of family 2 and comparing the ratio of black and red on the whole coat to the same ratio for a certain area of the coat. The ratio of black to red in the two large lines of family 2 was

almost equal, although there is a large difference with respect to the amount of white, as has been mentioned previously. The ratio of black to red is slightly larger with males than with females. The tabulation of shoulder and hip spots was not segregated as to sex, but the ratio of the sexes combined is not greatly different from the ratio for the whole coat. Shoulder spots and hip spots show a greater tendency to red. This tendency to red is still greater in the nose spots, where the ratio for the sexes combined for family 2 is 1 : 2.5 and for 2N is 1 : 3. These color relations are shown in Table 6.

TABLE 6.—*Color relations of guinea pigs in lines of family 2 and in experiment 2N*

PER CENT OF COLOR ON WHOLE COAT OF ANIMALS

Lines	Black		Red		White		Ratio black to red	
	Males	Females	Males	Females	Males	Females	Males	Females
	Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
2-8-4	18.364	13.630	27.051	18.766	55.457	68.756	1:1.47	1:1.38
2-9-7	7.722	6.140	11.308	8.246	82.715	87.590	1:1.46	1:1.34

PER CENT OF ANIMALS WITH DIFFERENT COLORED SPOTS, FAMILY 2 AND EXPERIMENT 2N

Part of body	Black	Red	White	Ratio black to red
	Per cent	*Per cent*	*Per cent*	*Per cent*
Right shoulder	13.89	20.34	65.77	1:1.46
Left hip	12.69	20.64	66.68	1:1.63
Nose, family 2	2.68	6.56	90.77	1:2.45
Nose, 2N	3.55	10.65	85.80	1:3.00
Tail, family 2	2.94	3.98	93.08	1:1.35

TYPES OF MATINGS

The matings that have produced nose-spot animals in family 2 and in experiment 2N have been classified as to parentage of the animals into groups according to the color of the nose spot, or whether there was no spot at all. Table 7 shows the percentage of animals of each class born in the different types of matings.

The absence of definite hereditary results may be explained when the genetic factors which determine the color pattern of family 2 are considered. The pattern concerned is known as the tricolor pattern, i. e., it consists of black, red, and white. Two genetic factors are involved, e^p, the factor for partial extension, which determines the presence of red spots on an otherwise solid black coat, or complete extension, *E*, and *s* the piebald factor which determines white spots on an otherwise solid colored coat, *S*. These factors are homozygous (e^p e^p *ss*) in the inbred stock. They do not, however, determine the amount of color or its location, for the amount of color varies greatly in the two lines of family 2, as has been pointed out before, and even in individuals of the same litter there is great variation. The correlation of amount of white on litter mates of this family is about 0.16 ± 0.026, being about equal in both lines, whether the correlation is between brothers, sisters, or between brothers and sisters.

TABLE 7.—*Types of matings and percentage of young produced, family 2 and experiment 2N*

Type of mating	Number of matings	Family 2					
		Black nose spot		Red nose spot		No nose spot	
		Number	*Per cent*	*Number*	*Per cent*	*Number*	*Per cent*
Black with black	1	1	4.35	0	0	22	95.65
Black with red	3	4	10.25	4	10.25	31	79.49
Black with normal	11	4	1.91	30	14.35	175	83.73
Red with red	2	2	7.69	3	11.53	21	80.77
Red with normal	38	22	4.71	55	11.76	390	83.51
Normal with normal	107	78	4.46	180	10.29	1,491	85.25
Both parents nose spot	6	7	7.95	7	7.95	74	84.09
One parent nose spot	49	26	3.85	85	12.57	565	83.58
All classes	162	111	4.41	272	10.82	2,130	84.75

Type of mating	Number of matings	Experiment 2N					
		Black nose spot		Red nose spot		No nose spot	
		Number	*Per cent*	*Number*	*Per cent*	*Number*	*Per cent*
Black with black	3	0	0	4	7.55	49	92.45
Black with red	21	10	3.66	28	10.26	235	86.08
Black with normal	1	2	8.33	4	16.67	18	75.00
Red with red	20	10	4.33	25	10.82	196	84.85
Red with normal	3	1	2.86	5	14.28	29	82.86
Normal with normal	2	0	0	3	9.38	29	90.62
Both parents nose spot	44	20	3.59	57	10.23	480	86.18
One parent nose spot	4	3	5.08	9	15.25	47	79.66
All classes	50	23	3.55	69	10.65	556	85.80

WORK OF OTHER INVESTIGATORS ON NOSE SPOT

MacCurdy and Castle (2) in their selection for nose spot obtained somewhat different results from those of the present writer. They increased appreciably the occurrence of nose spot in their stock. From a nose spot male mated with different kinds of females, they obtained 50 per cent of the young with nose spots. In matings of the descendants of this male with nose-spot females, 39.8 per cent of the young had nose spots. In matings in which none of the parents bore a nose spot, 16.3 per cent of the young had nose spots. These experiments showed an increase in the amount of nose-spot young with an increase in the amount of nose-spot ancestry, and strongly suggested the possibility of a still further increase by continued selection. MacCurdy and Castle had not obtained such an increase, however, at the time of the publication of their results. Their results differed further from those obtained in the Bureau of Animal Industry experiment in the amount of white on the animal. In every case the young with nose spots had fewer other spots on the body than the young without nose spots. While the data of MacCurdy and Castle do not take into account the size of the spots, and thus can not be expressed in percentage of white or colored area, the fact that there is a larger average of other colored areas in the case of the animals without nose spot suggests that these animals also have less white than the nose-spot individuals. This is directly opposite to the result found in the Bureau of Animal Industry experiment.

OCCURRENCE OF TAIL SPOT

Another interesting pattern that has been studied along with nose spot is *tail spot*. This pattern consists of a small patch of color covering the tail area. Animals which have shown white on one side of the mid-dorsal line and color on the other, or which have color extending across the whole rump on both sides have not been included as tail-spot animals. The five inbred families and the animals in experiment B have been included in this study. The percentage occurrence of tail spot is given in Table 8.

TABLE 8.—*Tail spotting in families of guinea pigs*

Stock	Total number of animals	Number of tail-spot animals	Per cent of tail-spot animals
Family 2 (early lines)	752	80	10.64
2-8-4 line	1,812	51	2.81
2-9-7 line	1,510	150	9.93
Total, family 2	4,148	287	6.92
Total, family 13	5,118	60	[a] 1.17
Total, family 32	3,695	16	[a] .43
Total, family 35	3,806	69	[a] 1.81
Total, family 39	1,978	93	[a] 4.70
Experiment B	5,697	229	4.02

[a] The average for families 13, 32, 35, and 39 is 1.63 per cent.

A condition very similar to that found in the nose-spot study is here shown. Different lines of family 2 show a difference in the percentage of tail spots produced, and the family as a whole produces more than any other of the inbred families, or than occur in the control experiment B. The average for the four inbred families, besides family 2, is 1.63 per cent.

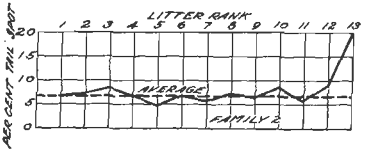

FIG. 5.—Percentage of tail spot by litter rank

In the study of family 2 by lines it has been found that there is a similarity to the nose-spot condition. The early lines produced 10.64 per cent of tail spot animals, or about the average of line 2–9–7. At the point where the two large branches emerge a difference in the amount of tail spotting appears, but the difference is in an opposite direction to that of the nose spotting. Line 2–8–4, which produces the most nose spot produces the fewest tail spots, only 2.8 per cent, and the low nose-spot-producing line, 2–9–7, produces the most tail spots, 9.9 per cent. This rather peculiar result makes it appear as though an animal does not have color on both extremities at once although several animals have been observed that had both nose spot and tail spot. At 2–12–8 a rise in percentage of tail spot is seen, persisting in the other lines that have descended from this mating. This is comparable with the rise in percentage of nose spot animals in the 2–15–6 and 2–18–1 sublines. This 2–12–8 line with the line descending from it produces a percentage of 11.45 tail-spot indi-

viduals, while the line 2–9–7 up to the beginning of 2–12–8 produced but 6.49 per cent, showing an increase of nearly 5 per cent. (Fig. 1.)

In studying the relations between tail spot and other characters it has not been considered necessary to go into detail as much as was done in the case of nose spot, for the relations appear practically the same. (Figs. 5 and 6.) Curves showing the percentage of young with nose spot born in a certain month agree very closely with the total percentage of tail-spot young born. Likewise, curves showing the percentage of tail-spot young born in a certain litter compare closely with the total percentage of tail-spot young born. Weights have not been considered, since in the discussion of nose spot it was shown that very little difference existed between the two large lines of family 2. It seems reasonable to expect the same with the tail-spot condition. Likewise, mortality has not been considered.

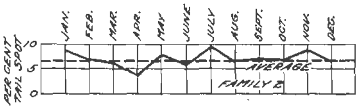

FIG. 6.—Month of birth of tail-spot guinea pigs

The relation between sex of the animal and color of the spot seems rather important to consider.

TABLE 9.—*Number and percentage of male and female guinea pigs having black or agouti and red, yellow, or cream tail spots*

Stock	Guinea pigs having black or agouti tail spots				Guinea pigs having red, yellow, or cream tail spots				Total number of guinea pigs
	Male		Female		Male		Female		
	Number	*Per cent*	*Number*	*Per cent*	*Number*	*Per cent*	*Number*	*Per cent*	
Family 2	59	20.56	63	21.95	80	27.87	85	29.62	287
Family 13	13	21.67	24	40.00	12	20.00	11	18.33	[a] 60
Family 32	4	25.00	7	43.75	1	6.25	4	25.00	16
Family 35	15	21.74	30	43.48	6	8.70	18	26.09	69
Family 39	17	18.28	55	59.14	11	11.83	10	10.75	[a] 93
B	51	22.27	87	37.99	44	19.21	47	20.52	229
Total	159	21.09	266	35.28	154	20.42	175	23.21	754
	Male and female				Male and female				
	Number		*Per cent*		*Number*		*Per cent*		
Total, by color	425		56.37		329		43.63		
	Male (all colors)				Female (all colors)				
	Number		*Per cent*		*Number*		*Per cent*		
Total, by sex	313		41.51		441		58.49		

[a] In family 13 there were 9 males and 9 females which had white tail spots surrounded by a colored area, and in family 39 there were 17 males and 10 females similarly colored. These have not been included in the tabulation.

In family 2 an excess of red tail spots (57.49 per cent red to 42.51 per cent black) was found. This does not approach, however, the excess of reds that was shown in nose spot, the ratio here being 1: 1.35 for both sexes combined. In the case of sex, there is a considerable excess of females with tail spots (58.49 per cent females to 41.51 per

cent males), whereas with nose spotting there was nearly equality between the sexes, 48.30 per cent females to 51.70 per cent males in family 2 and 2N. The large inequalities in tail spotting may be due to the smaller number of individuals involved, still there were only about 100 more nose-spot animals than tail spot, so that differences due to numbers should not be great.

No matings have been made selecting for tail spot, but it is reasonable to suspect that results not differing greatly from those found in experiment 2N would be obtained. The same process of brother-sister mating has been carried out in both lines of family 2, and any matings of tail-spot animals have thus been made entirely at random. But since in the inbreeding process a constantly higher degree of homozygosis is being reached from generation to generation, and selection is taking place automatically, further selection from closely related individuals would not alter materially the type already present.

AREAS OF PIGMENTATION

Allen (*1*) has pointed out that animals have regularly pigmented areas. In most birds and mammals there are five of these centers on each side and a median one on the forehead. These patches are independent of one another and there may or may not be bilateral symmetry. The pigment areas are the coronal, or crown patch, aural or ear patch, nuchal or neck patch, scapula or shoulder patch, pleural or side patch, and sacral or rump patch. In most animals the rump patches remain fused dorsomedially and give the appearance when reduced of a single median patch at the base of the tail. The ear patch seems to be the most persistent, agreeing in this respect with the observation of MacCurdy and Castle (*2*), who say that in guinea pigs spots occur more frequently on the head than on any other part of the body.

These results conform closely with those obtained in studies of the stock of guinea pigs of the Bureau of Animal Industry. No definite investigation has been made of the regions of pigmentation, but observation of the animals clearly shows this regional division, and in many instances it is very definitely marked.

It also appears that the spots are independent of one another, for there is usually a noticeable lack of bilateral symmetry, and the pigmented areas even on the same side are not usually of the same color. The persistence of the head spots also seems to be in agreement, for often animals are found with no color on the body except a small patch on the ear or around the eye or sometimes a few colored hairs between the ears.

SUMMARY

In experiments with guinea pigs by members of the Bureau of Animal Industry, one family (family 2) used in the inbreeding experiments was found to produce a number of nose-spot and tail-spot animals.

This family became divided into two large lines in the eighth and ninth generations, one line producing more than 14 per cent of nose-spot young, the other a little more than 3 per cent.

The line producing a high percentage of nose spot produced a low percentage of tail spot—a little less than 3 per cent, while the other line produced nearly 10 per cent of tail spot.

Other inbred families produced slightly more than 1 per cent nose-spot and tail-spot young. In crosses between family 2 and four other inbred families slightly more than 3 per cent of nose spot was produced. Crosses of the high and low producing lines of family 2 with family 13 showed slightly more nose spots from the high line than from the low, and a slight increase in the F_2 and F_3 generations over the F_1.

A selection experiment, 2N, did not produce more nose-spot animals than the high-producing line of family 2. The amount of nose-spot ancestry had little effect on the number of nose-spot young, except that there was a lower percentage of nose spot when none of the parents, grandparents and great-grandparents had nose spot.

There is little or no relation between the nose-spot character and birth weight, size of litter, mortality, season of birth, sex, or litter rank in which such pigs were born. The difference in the amount of white on nose-spot animals and those without it can be accounted for by the spot itself.

Nose spot and tail spot appear to be inherited, but the inheritance can not be explained by simple Mendelian factors. Different types of matings produced all types of young in about the same proportions.

In family 2 and 2N there was an excess of red spots on the nose, tail, right shoulder, and left hip. This excess is in about the same ratio as red to black on the whole coat area of the animal, except that the nose shows a greater tendency to be red, if it is colored at all, than other portions of the animal.

The necessary factors for the tricolor pattern ($e^p e^p ss$) being present, the location of the spots themselves appears to be determined by local conditions in the developing skin. These conditions are very irregular in the guinea pig, but a slight heredity of the tendency of the colors to develop in certain spots exists in certain lines.

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THE EFFECT OF SUCCESSIVE GENERATIONS OF YEAST ON THE ALCOHOLIC FERMENTATION OF CIDER [1]

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INTRODUCTION

With the rapidly increasing demand for unfermented fruit juices during recent years much interest has been aroused in methods of manufacture. Although fruit juices are very attractive when first pressed they soon lose their attractiveness by yeast development, and consequently can not be handled or stored properly without being subjected to some process to check fermentation. Any treatment subsequent to pressing seems to injure more or less the delicate flavors and aromas of the juices. Filtering may improve the appearance but it does not improve the natural flavor. Chemical preservatives added in certain proportions may check fermentation but they often impart disagreeable flavors to the juices. Moreover, the use of such preservatives is of questionable propriety, and, as their presence is generally looked upon with suspicion by the public, their use is gradually decreasing. Pasteurization, although free from some of the objectionable features of the chemical preservatives, often gives a cooked taste and checks fermentation only so long as the treated juices are kept out of contact with the air. Refrigeration approaches perhaps most nearly the ideal of preserving the natural flavors of the juices, but it is an expensive process and unless the temperature is constantly kept at or near the freezing point of the juices fermentation starts readily and makes the product unfit for unfermented beverages. Since both pasteurization and refrigeration fail to produce unfermentable products, the objection that the juices so treated may be used for unlawful purposes remains unsolved. With the exception of the rather unsatisfactory use of chemical preservatives, there is yet no reliable method for the preparation of unfermentable fruit juices. In view of the rapidly growing demand for unfermented beverages it is evident that there is an urgent need for some practical method whereby clear, attractive, unfermentable fruit juices can be prepared with the least possible loss of natural flavors and aromas. There is still much to be learned about the fermenting organisms and their mineral food requirement. Careful research in this direction will undoubtedly bring to light much valuable information and may open the way to a successful solution of the unfermented-beverage problem. The work described in this paper is a preliminary study dealing with the application of some of the known basic factors in fermentation in an attempt to open the way to the development of some practical method of making unfermentable fruit juices without the use of chemical preservatives.

[1] Received for publication May 23, 1928; issued September, 1928. Published with the approval of the Director of the Washington Agricultural Experiment Station as Scientific Paper No. 148, College of Agriculture Experiment Station, State College of Washington.

SCOPE OF THE INVESTIGATION AND METHODS OF PROCEDURE

It has frequently been observed that bacterial cultures develop very poorly or refuse to grow at all in bouillon media that have once served for the growth of these same bacteria. It seems that the by-products accumulated by the first generation make the media unsuitable for the growth of successive generations. This observation is not limited to bacteria but applies equally well to yeast. When alcoholic fermentations are checked by rising temperatures, which are normal in active fermentation, it is more difficult thereafter to resume the fermentation in the normal way. The reasons generally ascribed for this behavior are that the increased temperature resulting from very active fermentation kills many of the active yeast cells and that the by-products of these dead cells retard or prevent the maximum development of the new generation. It is this assumption perhaps which induced Boulard[2] to study the phenomenon with the end in view of using it methodically in the control of alcoholic fermentations of wines. By growing successive generations of yeast in wines he succeeded in rendering them totally unfermentable long before all the fermentable sugars were used up. According to his expression these wines were "immunized" or "vaccinated", against yeast and also against spoilage bacteria. Although the causes of this so-called immunization or vaccination have not been studied and are as yet insufficiently understood, it is presumed that the accumulated by-products of preceding generations of yeast are toxic to the extent that further yeast or bacterial development is altered or even prevented. It is also thought that successive generations of yeast soon exhaust certain essential food elements so completely that the medium is no longer capable of supporting yeast and bacterial growth. These two assumptions constituted the main object of this study. Cider pressed from cull apples of mixed varieties was used in the experiments.

The toxicity studies were carried out by placing definite amounts of this cider in Erlenmeyer flasks, inoculating them with a heavy suspension of yeast culture, and incubating them at 25° C. Active fermentation was checked at will by heating the inoculated cider at 45° C. for one hour. As this treatment killed many of the active yeast cells, reinoculation following each heating was necessary. The process was repeated until the yeast refused to develop. Three or four inoculations were usually sufficient to accomplish this end.

Because of the well-known fact that cider is generally very low in nitrogen and phosphorus, both of which are believed to be indispensable for the growth and reproduction of yeast, the study of the possible depletion of certain food elements by yeast growth was limited to quantitative and qualitative determinations of these two elements. Determinations of alcohol and acetic acid were also made periodically but only for the purpose of following the course of their formation and of ascertaining how low their percentages could be kept during the process.

All the quantitative chemical determinations were made according to the official methods of the Association of Official Agricultural

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[2] BOULARD. SUR UN PROCÉDÉ PERMETTANT D'ARRETER À VELONTÉ LES FERMENTATIONS A N'IMPORTE QUEL MOMENT. Compt. Rend. Acad. Agr. France 12: 615–620. 1926.

Chemists.[3] The qualitative tests for nitrites were made with Trommsdorf's reagent, those for nitrates with diphenylamine reagent, those for ammonia with Nessler's reagent, and those for orthophosphates with ammonium molybdate reagent followed by reduction with tin metal according to the method of Spurway.[4]

EXPERIMENTAL DATA

TOXICITY AND ALCOHOL PRODUCTION OF SUCCESSIVE GENERATIONS OF YEAST AND EFFECT ON NITROGEN AND PHOSPHORUS CONTENT OF CIDER

Three 2-liter Erlenmeyer flasks were filled with 1,500 c. c. of freshly pressed cider and two of them were heated at 45° C. for one hour and then cooled to 25°. Since heating caused considerable precipitation in the cider, the precipitate was first allowed to settle and was then separated by decantation. The supernatant liquid and sediment were analyzed separately for nitrogen and phosphorus. As the concentration of sediment naturally varied in different decantations, the results of the analysis of the various sediments could not be expected to agree very closely, but the analysis was made only to determine the approximate proportion of these elements in the two separated parts. When the analysis was completed the sediment was rejected but the flasks with the supernatant liquid of the heated cider and that with the unheated fresh cider were inoculated with 2 per cent of a heavy suspension of *Saccharomyces valesiacus* (Osterwalder) at its maximum activity in sterilized cider. The inoculum was thoroughly mixed with the cider in the flasks and allowed to develop at 25° until the fermentation became active as shown by the appearance of foam bubbles on the surface of the liquid. The three flasks were then heated at 45° for one hour, the heating causing once more considerable precipitation which was allowed to settle before being separated by decantation. The analyses for nitrogen and phosphorus of the supernatant liquid and sediment were made separately as before. Reinoculations with consequent heatings and analyses were repeated three times, resulting in progressively smaller amounts of precipitated material and a considerable decrease in yeast development each time. A fourth inoculation was made in which the amount of inoculum was twice that which was used previously. The cider at that time was already clear and remained so even after 24 hours' incubation at 25°. Neither was there the slightest indication of any yeast development observable by macroscopic means during that time. The results of the various analyses obtained up to this stage are reported in Table 1.

The data indicate that cider is very low in nitrogen and phosphorus, that a large part of these elements was easily precipitated by a temperature of 45° C., and that most of that remaining was used up by the first generation of yeast. However, the removal of the last traces of ammonia nitrogen and orthophosphates proved to be extremely difficult as indicated by the results of the qualitative analyses. A positive test for these substances was secured as long as the experiment was in progress, but the nitrogen was never found in the nitrite or nitrate forms in any of the experiments.

[3] Association of Official Agricultural Chemists. Official and tentative methods of analysis. Compiled by the committee on editing methods of analysis. Revised to July 1, 1924. Ed. 2, 535 p., illus. Washington, D. C. 1925.

[4] Spurway, C. H. Test soils for water-soluble phosphorus. Mich. Agr. Expt. Sta. Quart. Bul. 9: 64–65. 1926.

TABLE 1.—*Analysis of cider and sediment in the first experiment at different stages of the treatment*

Treatment	Quantitative analysis in percentages						Qualitative analysis [a]			
	Supernatant liquid				*Sediment*		*Supernatant liquid*			
	C_2H_5OH by weight	Acidity in terms of CH_3COOH	*Nitrogen*	Phosphorus	*Nitrogen*	Phosphorus	Nitrites	Nitrates	Ammonia	Orthophosphates
Cider unheated before first inoculation.										
Fresh cider	0.00	0.40	0.0068	0.0028			−	−	+	+
First inoculation			.0		0.035		−	−	+	+
Second inoculation			.0	.0	.0056	0.0051	−	−	+	+
Third inoculation	.56	.44	.0		.0002		−	−	+	+
Cider heated before first inoculation:										
Fresh cider heated at 45° C. for 1 hour	.00	.39	.0056	.0013	.028	.0041	−	−	+	+
First inoculation			.0		.039		−	−	+	+
Second inoculation			.0	.0	.013	.0082	−	−	+	+
Third inoculation	.53	.44	.0	.0	.0024	.00	−	−	+	+

[a] + = positive test; − = negative test.

The effect of successive generations of yeast was shown by a cell development which was in inverse proportion to the number of generations. The third generation grew very poorly and the fourth failed to show any signs of growth for some time at least. By carefully checking the development of each generation as soon as a few foam bubbles appeared on the surface of the cider, it was possible to limit the formation of alcohol to as small amounts as 0.56 and 0.53 per cent. Although it is true that even the smaller amount is slightly in excess of the limits permitted by the law applying to unfermented beverages, probably by more carefully checking the development of the yeast this excess could be reduced sufficiently to comply with the requirements of the law.

The change in total acidity during the process was insignificant and the results in general were most gratifying in every respect up to this point.

At the end of 24 hours' incubation following the fourth inoculation plate counts for the number of living yeast cells in the treated ciders were made on plain agar supplemented with 10 per cent cider. This modified plain agar was used throughout this study because the yeast developed better on it than on the plain agar. The plate counts were repeated after 5 days' incubation and also after 10 days' incubation. The results together with those of the qualitative tests for ammonia nitrogen and orthophosphates are recorded in Table 2.

TABLE 2.—*The determination of active yeast cells, ammonia, and orthophosphates in cider at different stages of incubation after the fourth yeast inoculation in the first experiment*

Treatment	Living yeast cells per cubic centimeter [a]	Qualitative analysis [b]	
		Ammonia	Orthophosphates
24 hours after fourth inoculation	1,760,000	+	+
After 5 days' incubation at 25° C	950,000	+	+
After 10 days' incubation at 25° C	1,600,000	+	+

[a] Each figure in this column is the average of counts taken from 4 plates.
[b] The plus sign indicates positive tests.

In spite of the fact that there was no visible change in the cider during the first few days following the fourth inoculation the data show that the yeast cells seemed to remain alive. At least they did not die at any abnormal rate, nor did they develop to any noticeable extent. On the other hand, bacterial growth, probably resulting from air contamination, seemed to make some progress, for on the fifth day of incubation a very light film was plainly distinguishable on the surface of the cider. On the sixth day some activity, apparently caused by the production of gas, was noticeable, and on the seventh day it was quite evident that at least some of the yeast cells were active. The plate count on the tenth day bore out this evidence by giving a decided increase in numbers of yeast colonies over those of the fifth day. Evidently the cider was not permanently unfermentable nor was it totally immune to bacterial growth. It was thought that the generations of yeast had perhaps not been given sufficient time to develop the necessary amount of toxic substances and so it was decided to repeat the experiment in a somewhat modified way.

In the second experiment two 4-liter flasks were filled with 3.5 liters of freshly pressed cider and heated for one hour at 45° C. The coagulated material resulting from the heating was allowed to settle and was then separated by decantation. The supernatant liquid and sediment were analyzed separately for nitrogen and phosphorus as in the foregoing experiment. One of the flasks containing the supernatant liquid was inoculated with 2 per cent of a heavy suspension of the natural cider flora at its maximum activity in cider and the other with a heavy suspension of *Saccharomyces valesiacus* as in the first experiment. The procedure from this point on was similar to that already described except that the development of the yeast, and consequently the fermentation, was allowed to continue a longer time for each generation before being checked by heating.

TABLE 3.—*Analyses of cider and sediment in the second experiment at different stages of the treatment*

Treatment	Quantitative analysis in percentages						Quantitative analysis [a]			
	Supernatant liquid				Sediment		Supernatant liquid			
	C_2H_5OH in weight	Acidity in terms of CH_3COOH	Nitrogen	Phosphorus	Nitrogen	Phosphorus	Nitrites	Nitrates	Ammonia	Orthophosphates
Heated fresh cider	0.0	0.43	0.0014	0.0029	0.0196	......	−	−	+	+
Cider inoculated with natural yeast flora:										
First inoculation	.0	......	.0	......	.093	......	−	−	+	+
Second inoculation	......	......	.0	.0	.101	......	−	−	+	+
Third inoculation	1.6	.42	.0	......	.0	......	−	−	+	+
Cider inoculated with S. valesiacus:										
First inoculation	.0	......	.0	......	.105	......	−	−	+	+
Second inoculation	......	......	.0	.0	.098	0.0105	−	−	+	+
Third inoculation	1.6	.43	.0	......	.0	......	−	−	+	+

[a] + = positive test; − = negative test.

At the fourth reinoculation the flasks were incubated at 25° C. and plate counts for yeast were made 24 hours later. The cider was then bottled in pint bottles and sealed by means of metal caps. Two

bottles taken from the cider inoculated with the natural flora and two from that inoculated with *Saccharomyces valesiacus* were pasteurized for 30 minutes at 80° C. and after cooling were placed in storage in the 25° incubator with the rest of the bottles. Two months later and again four months later agar plates were prepared for yeast counts of both the pasteurized and unpasteurized bottles, and at the same time determinations for alcohol, nitrites, nitrates, ammonia, and orthophosphates were made. The data recorded in Tables 3 and 4 show that the results of this experiment were quite similar to those of the first experiment.

TABLE 4.—*Determination of active yeast cells, ammonia, orthophosphates, and per cent of alcohol in cider at different periods of incubation after the fourth inoculation with yeast in the second experiment*

Treatment	Living yeast cells per cubic centimeters[a]	C_2H_5OH	Nitrites	Nitrates	Ammonia	Orthophosphates
Cider inoculated with natural yeast flora:						
24 hours after fourth inoculation	300,000	1.60	−	−	+	+
After 2 months' incubation at 25° C	9,850,000	1.60	−	−	+	+
After 4 months' incubation at 25° C	1,700,000	2.60	−	−	+	+
Cider inoculated with S. valesiacus:						
24 hours after fourth inoculation	930,000	1.60	−	−	+	+
After 2 months' incubation at 25° C	240,000	1.87	−	−	+	+
After 4 months' incubation at 25° C	25,000	2.70	−	−	+	+
Cider inoculated with natural yeast flora of cider and that inoculated with S. valesiacus, but Pasteurized 24 hours after fourth inoculation:						
24 hours after fourth inoculation	0	1.60	−	−	+	+
After 2 months' incubation at 25° C	0	1.60	−	−	+	+
After 4 months' incubation at 25° C	0	1.10	−	−	+	+

[a] Each figure in this column is the average of counts taken from 4 plates, duplicate plates being prepared from 2 bottles.

There was a natural increase in alcoholic content due to the extra time each generation of yeast was allowed for development. However, this greater development of the several generations of yeast did not have the effect that was hoped for, namely, that of making the cider permanently unfermentable. Only the pasteurized bottles proved to be inactive. In all the other bottles fermentation took place slowly but continuously during the four months the cider was kept in storage. The renewed activity of the yeast probably began about the second week of incubation as in the first experiment. Although fermentation progressed to a certain extent the conditions for growth were never favorable. This was especially true for *Saccharomyces valesiacus*, as proved by the rapidly decreasing number of living cells during the four months of incubation. The yeast of the natural flora of the cider, probably composed of more than one species, behaved somewhat differently. The percentage of inoculum at the fourth inoculation being the same for the two yeasts, the concentration of the living cells might be expected to be similar also. But this was not the case, for the plate count showed that the number of living yeast cells was much smaller in the cider inoculated with the natural flora than in that inoculated with *Saccharomyces valesiacus*. However, as the time of incubation advanced the concentration of living cells became greater in the former than in the latter, indicating

that certain species of yeast are more resistant to the effect of their by-products than others. But, on the other hand, the amount of alcohol produced by the two kinds of yeast was approximately the same though the number of living cells varied widely. The reason for this may be explained by the fact that the members of the species *Saccharomyces valesiacus* which are known for their high alcohol production are probably more efficient alcohol producers than the members of mixed species generally found in cider. Furthermore, the number of the growing cells which appeared on the agar plates are not necessarily an indication that all these cells were active under the extremely unfavorable conditions of the cider. Many may have been inactive in the cider and the alcohol produced may have been the result of the activity of comparatively few cells in both cases.

It is interesting to note that the number of living cells in both kinds of yeast declined rapidly during the last two months of incubation. It may be that the increasing pressure resulting from continued CO_2 production in the sealed bottles was a contributing factor. Or possibly the mineral food shortage became more intense as the fermentation progressed. Either or both of these factors may reasonably be expected to exert some influence on the development of living cells. In order to ascertain to what extent they were responsible for the conditions in the bottled cider the following experiment was prepared:

Fourteen 250 c. c. cotton-plugged Erlenmeyer flasks were sterilized and after being cooled 12 of them received 100 c. c. each of the cider that had been pasteurized at the fourth inoculation of the previous experiment and was known to be free of living yeast cells. The two remaining flasks received 100 c. c. each of sterilized freshly pressed cider. The flasks were arranged in 2 series of 7, each series containing 1 flask of the sterilized fresh cider and 6 of pasteurized cider that had been treated by successive generations of yeast. One series was inoculated with 1 c. c. quantities of cider from the sealed bottles treated with the natural yeast flora of cider in the previous experiment and the other series was inoculated with 1 c. c. quantities of cider from the sealed bottles treated with *Saccharomyces valesiacus* in the previous experiment. All this was done aseptically to eliminate possible errors from contaminations. The cotton plug in each flask was then replaced by a sterilized rubber stopper through which one end of a glass tube bent at right angles was passed. The other end was connected to a similar tube inserted in N/1 KOH in an absorption tower for the purpose of collecting the CO_2 produced. The two series were further treated as follows:

The flask containing the sterilized fresh cider and the first flask containing the pasteurized treated cider in each series were not treated and served as controls.

The second flask of pasteurized treated cider in each series received 0.1 per cent peptone.

The third flask of pasteurized treated cider in each series received 0.05 per cent of ammonium nitrate.

The fourth flask of pasteurized treated cider in each series received 0.1 per cent secondary potassium phosphate.

The fifth flask of pasteurized treated cider in each series received 0.05 per cent ammonium nitrate and 0.1 per cent secondary potassium phosphate.

The sixth flask of pasteurized treated cider in each series received 0.1 per cent peptone in addition to 0.05 per cent ammonium nitrate and 0.1 per cent secondary potassium phosphate.

The added materials were thoroughly mixed with the cider in the flasks and the two series, properly arranged and connected, were incubated at 25° C. for two weeks. At the end of the incubation period each flask was thoroughly shaken to expel the gases absorbed in the cider and the CO_2 collected in the absorption towers was determined by double titration, thymol blue and brom phenol blue being used as indicators. The data obtained are given in Table 5.

TABLE 5.—*The effect of peptone and mineral food elements on the activity of yeast in cider rendered temporarily unfermentable by successive generations of yeast*

Treatment	Per cent of alcohol and milligram of CO_2 in 100 c. c. after 2 weeks' incubation at 25° C.			
	Inoculated with natural flora from treated cider		Inoculated with S. valesiacus from treated cider	
	CO_2	C_2H_5OH	CO_2	C_2H_5OH
Pasteurized treated cider	57		101	
Freshly pressed sterilized cider	5,394	5.61	5,614	5.94
Pasteurized treated cider+0.1 per cent peptone	2,824	5.53	2,270	5.40
Pasteurized treated cider+0.05 per cent NH_4NO_3	2,626		1,408	
Pasteurized treated cider+0.1 per cent K_2HPO_4	272		365	
Pasteurized treated cider+0.05 per cent NH_4NO_3+0.1 per cent K_2HPO_4	2,930	4.96	2,345	5.29
Pasteurized treated cider+0.05 per cent NH_4NO_3+0.1 per cent K_2HPO_4+0.1 per cent peptone	3,066	5.61	3,014	5.37

The active fermentation in the sterilized freshly pressed cider offers conclusive evidence that both kinds of yeast resumed their normal activity when exposed to the proper media. The data also indicate that the pressure existing in the sealed bottles as a result of CO_2 production had little or nothing to do with the rapidly decreasing number of living cells in the sealed bottles during the last two months of incubation. The addition of phosphates alone did not increase the activity of either kind of yeast, but the addition of ammonium nitrate caused a slight increase, showing that nitrogen was a limiting factor. When both ammonium nitrate and secondary potassium phosphate were added there was a decided increase in CO_2 production, indicating that one of the causes responsible for the limited activity of the yeast in the sealed bottles was lack of mineral food. The reason that more CO_2 was produced by the addition of peptone than by the addition of ammonium nitrate was perhaps that the organic form of nitrogen was better assimilated and more suitable for yeast growth than the inorganic form or that traces of phosphates were present in the peptone. The presence of traces of phosphates was indicated by a positive test for orthophosphates.

If the results of this experiment alone are considered it might be assumed that the extremely limited action of yeast in cider in which several generations had developed was chiefly due to lack of food. This assumption is supported by the fact that the addition of suitable forms of nitrogen and phosphorus caused a spontaneous and vigorous resumption of fermenting activity which resulted in a yield of alcohol approaching the normal amount in cider. But opposed to this assumption is the fact that the qualitative test for ammonia and

orthophosphates was, as far as could be observed, identical at the beginning and at the end of the incubation period, while the number of living cells seemed to increase at the beginning but showed a rapid decrease at the end of the incubation period. Granting that the food supply was constant throughout the incubation period, it seems that the number of living cells should also have been approximately the same during that period if the food supply was the only limiting factor. It would therefore appear that other causes were partly responsible. The accumulation of toxic products, generally assumed to be derived from the by-products of metabolism or from disease conditions, but as yet poorly understood because of their complexity and the lack of adequate methods, was perhaps a contributing cause. It is true that the effect, if there was any, was ultimately of minor consequence, as is shown by the final high yield of alcohol, but whatever effect there was may have been counteracted totally by enzymic autofermentation. These factors will be considered more fully in a succeeding experiment.

Two important observations not shown by the data presented, but valuable from the standpoint of manufacture, were made in these experiments and should not be overlooked. One is that the heatings required in the process caused complete coagulation of the material in suspension, resulting in a clear and attractive cider without the usual slow and tedious operation of filtering. The other is that as far as could be ascertained by taste the heatings at this low temperature did not affect to any perceptible degree the natural flavor of the cider. The first is very important from the standpoint of cost of manufacture and the second is valuable from the standpoint of quality of the product.

EFFECT OF THE FILTRATE OF THE TREATED CIDER ON ALCOHOL PRODUCTION AND ON THE DEVELOPMENT OF YEAST

Since yeast enzymes are able to ferment the same sugars that the living cells secreting them ferment, it is possible that some of the alcohol in the stored cider was caused by autofermentation of zymase freed by the disintegration of dead yeast cells. As the temperature used in heating the cider for the purpose of checking the growth of the several generations of yeast was too low to inactivate the enzymes, many of them might be liberated at each heating by maceration, thus giving them an unusual opportunity to exert considerable influence on the fermentation. The extent of this influence is difficult to determine correctly because these free enzymes are not easily obtained in a pure state. The filtering membranes of the porous candles generally used for this purpose may or may not permit their free passage, depending on the electrical charges of the filter. However, the pressure commonly used on these filters counteracts the effect of the opposite electrical charge of the filtering membrane to a certain extent and forces some of the enzymes through the openings irrespective of electrical charges. Thus approximate results may be obtained by the use of the porous candle. In this experiment a portion of each of the two differently treated ciders used in the second experiment was filtered through a sterilized N. Berkefeld candle. Duplicate 100 c. c. quantities of each filtrate were mixed with 100 c. c. of Buchanan solution previously sterilized in 250 c. c. Erlenmeyer flasks. The Buchanan solution was added to provide mineral food substances which might be lacking in the filtrates. Special emphasis

was given to soluble phosphates, as this substance seems to be necessary and is known to greatly accelerate the autofermentation of enzymes. According to Harden [5] autofermentation in media poor in phosphates may be increased from 10 to 150 per cent by the addition of suitable forms of phosphates. After thoroughly mixing the contents in the flasks, each flask was connected to an absorption tower in the manner described in the previous experiment and was incubated at 25° C. for two weeks. At the end of the incubation period the flasks were shaken vigorously to liberate any gases that might be absorbed in the solution and force them into the absorption towers to be taken up by the KOH solution. Determinations for CO_2 were made by the method previously used.

The amount of CO_2 formed was found to be so small as to be insignificant, indicating that autofermentation by enzymes was absent in the filtrates and supporting the assumption that the alcohol present in the treated ciders was probably the result of the usual activity of living yeast. As pointed out before, even this activity was very limited, as only about 1 per cent of alcohol by weight was formed during four months of incubation at the optimum temperature for yeast growth. Why the amounts of alcohol were approximately the same in both of the treated ciders irrespective of the wide variation in numbers of living yeast cells between that inoculated with the natural yeast flora and that inoculated with *Saccharomyces valesiacus* is not clear. It has many times been observed that certain individual cells of a given species of microorganism are able to perform their activities apparently unaffected in culture media which are toxic and prove to be decidedly destructive to the large majority of the cells. Likewise, certain cells of a given species have often been known to acquire a resistance to disease conditions, such as bacteriophage, to the point that they are able to grow and reproduce normally in the infected media while the majority of the cells are dissolved by the bacteriophage principle. Perhaps this same principle of adaptation or resistance is applicable to media with limited amounts of food supply. Thus it may be supposed that the majority of the yeast cells in the treated cider were unable to absorb sufficient food for growth and reproduction and finally died, while a few of the more resistant cells were able to adjust themselves to these unfavorable conditions and performed their normal activity successfully. Consequently, many of the yeast cells which were capable of growth and reproduction when plated out on favorable media were unable to perform their normal function of converting sugars into alcohol when exposed to the existing unfavorable conditions in the treated ciders. The result naturally followed that the alcohol formed was the product of the activity of comparatively few yeast cells. The fact that by far the larger part of the alcohol was produced during the last two months of incubation when the largest reduction of living cells was in evidence, seems to lend support to this supposition. That the limited activity of the yeast during the incubation period was mainly due to the limited food supply was demonstrated by the extent of the renewed activity resulting from the addition of suitable food substances and also by the final normal yield of alcohol. But, nevertheless, there is a possibility that accumulated toxic products or bacteriophage had some effect on the yeast development.

[5] HARDEN, A. ALCOHOLIC FERMENTATION. Ed. 3, p. 57-58. London, New York, [etc.], 1923.

The effect of the suspected toxic products of the treated cider on yeast development was determined by an experiment in which 10 per cent of the filtrates of the treated ciders was added to the decanted supernatant liquid of fresh cider heated at 45° C. for one hour. The filtrates were obtained as in the previous experiment and were added at the first inoculation together with the usual 2 per cent of inoculum. The filtrate obtained from the cider treated with the natural flora of yeast was added to the fresh cider inoculated with the natural flora of yeast, while the one obtained from the cider treated with *Saccharomyces valesiacus* was added to the fresh cider inoculated with *S. valesiacus*. Following this treatment the experiment was conducted exactly like the second experiment, but while it was in progress careful observations were made of the behavior of the fermentation and of the growth of yeast. The entire behavior proved to be similar in all respects to that of the second experiment and because of this similarity the data are not given. The results showed that the addition of 10 per cent of the filtrates did not have any retarding effect on the development of yeast.

Determinations for the bacteriophage principle were made by filtering through sterilized N. Berkefeld candles several samples of cider of the first and second experiment after the third and fourth inoculations. Quantities of 1 c. c. of these filtrates were added to bouillon and plain agar tubes containing 8 c. c. of the respective media. These were seeded with 1 c. c. of a heavy suspension of *Saccharomyces valesiacus* and the agar tubes were used to pour plates. The plates and the bouillon tubes were incubated at 25° C. for a week and were examined carefully at short intervals for any dissolving action of bacteriophage. The experiment was repeated several times, but at no time could any lytic or solvent action be observed either in the plates or in the bouillon tubes, thus indicating that bacteriophage was not present in the cider and consequently had no effect on the yeast development.

DISCUSSION

The results of the preceding experiments show the possibility of rendering cider temporarily unfermentable by growing successive generations of yeast and also the probability that the alcohol content produced during the process can be kept within the limits prescribed by the law for unfermented fruit juices. It was found that the factors chiefly responsible for the unfermentable condition of the cider are not the accumulated toxic products of preceding generations or the effects of disease conditions of the yeast itself, but the lack of nitrogen and phosphorus. The reason that the fermentation in the treated cider resumed a limited activity under optimum conditions for yeast growth after a week of apparent inactivity is probably due to the fact that the small traces of mineral food present in the cider were sufficient to support the growth and reproduction of a few yeast cells. That these traces of nitrogen and phosphorus did not seem to disappear with the subsequent limited yeast activity may be explained by the supposition that through the process of hydrolysis of the dead cells new supplies of available forms of these elements sufficient to maintain the life processes of a comparatively small number of cells were constantly being provided. Complete removal of nitrogen and phosphorus or even of phosphorus alone would very

likely result in a permanently unfermentable product. It is generally agreed that the development of yeast is impossible in the total absence of phosphorus, and Harden[6] states that fermentation by zymase should not occur in the total absence of phosphates.

In this study only partial success in removing either the nitrogen or the phosphorus was obtained, but it should be remembered that the work was preliminary and that further research may accomplish much. The results obtained seem to indicate that the best hope for success in this process lies in the total removal of phosphorus, because it prevents both fermentation and autofermentation. If this could be accomplished successfully the process would offer many obvious advantages. Manufacture would be simplified by eliminating the slow and tedious operations of filtering, but would nevertheless give an attractive, clear beverage possessing the natural flavor of the unprocessed freshly pressed cider. The elimination of the filtering operation would greatly reduce the cost of the initial capital outlay for machinery, thereby making possible the building of small plants at centrally located points and thus vastly diminishing the cost of transportation of the bulky raw material. In this way both manufacturer and producer would realize better returns, and a large percentage of the cull fruit now going to waste could be utilized in the form of a healthful refreshing beverage.

SUMMARY

A preliminary study was made of the effect of successive generations of yeast on the alcoholic fermentation and the beverage quality of cider.

The results showed that the nitrogen and phosphorus content of cider was low and that all but traces of each were readily removed by coagulation at a temperature of 45° C. and the growth of two or three generations of yeast.

Qualitative analyses indicated that nitrogen was at no time present in the nitrate or nitrite form, but was always present in small amounts in the form of ammonia. A positive qualitative test was at all times obtained for orthophosphates.

Clear cider, containing 0.53 per cent of alcohol and possessing the natural flavor of unprocessed, freshly pressed cider, was obtained in a temporarily unfermentable form by the growth of three generations of yeast.

The chief factors responsible for the temporary unfermentable condition of the cider were found to be the lack of nitrogen and phosphorus and not the effect of accumulated toxic products or of disease conditions of the yeast.

It was observed that the temporarily unfermentable cider was subject to a limited amount of fermentation after seven days of incubation at the optimum temperature for yeast and that this was probably due to the presence of traces of available nitrogen and phosphorus which were sufficient to maintain the life processes of a small number of yeast cells.

[6] HARDEN, A. Op. cit.

EFFECT OF VARIATION OF POTASSIUM AND CHLORINE IN A WHEAT RATION[1]

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INTRODUCTION

In a paper by Olson and St. John (*7*),[2] it was suggested that the addition of sodium to a ration based upon wheat was of greater benefit than the addition of potassium, and that the use of sodium salts in place of potassium salts in experiments previously reported by other investigators might have entirely altered the results obtained. Following this work a study was made of the effect of varying the amount of potassium in a ration based upon wheat. A study of the effect of the variation of chlorine in this same basal ration was also made. The white rat was the experimental animal used in all of this work. The present paper reports these studies and is the second progress report on the project dealing with the nutritive value of wheat.

REVIEW OF LITERATURE

Miller (*4*), using only 0.037 per cent of potassium in a synthetic diet, found that poor growth resulted. He believes that the minimum potassium requirement is between 0.055 and 0.144 per cent in such a ration. He states that the growth of rats can be greatly retarded by reducing the amount of potassium below approximately 1 per cent. He does not believe that sodium can replace potassium. A ration with a ratio of potassium to sodium of 14:1 is said to have no deleterious effect on the growth of young rats. Miller (*5*) found that the introduction of potassium salts into a synthetic diet caused immediate but temporary increase of sodium and chlorine excretion in the urine. He believes that the potassium requirements for animal development are abundantly satisfied in the ordinary ration.

Richards, Godden, and Husband (*10*) feel that the assumption that any ordinary ration will contain sufficient sodium and potassium is probably right. The addition of potassium citrate to a ration of cereal grain containing maize, oatmeal, barley, and blood meal fed to growing pigs lead to a decreased assimilation of nitrogen, phosphorus, and calcium. They feel that any adverse effect of large amounts of potassium is probably due to the depressing influence on the metabolism of these three elements and not to the impoverishment of the organism in sodium and chlorine.

Osborne and Mendel (*8*) report growth of rats on a synthetic ration containing 0.03 per cent potassium, although when both sodium and potassium were low, growth ceased. Their control diet contained 0.83 per cent of potassium. They also report completion of

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[2] Reference is made by number (italic) to "Literature cited," p. 63.

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growth on less than 0.04 per cent of chlorine. They quote Rosemann (*11*) as demonstrating that a diet deficient in chlorides leads to an insignificant reduction in the total chlorine content of the body. They suggest that the result is attributable to a husbanding of this element. Richards, Godden, and Husband (*10*) believe that the theory of chlorine impoverishment caused by high potassium intake

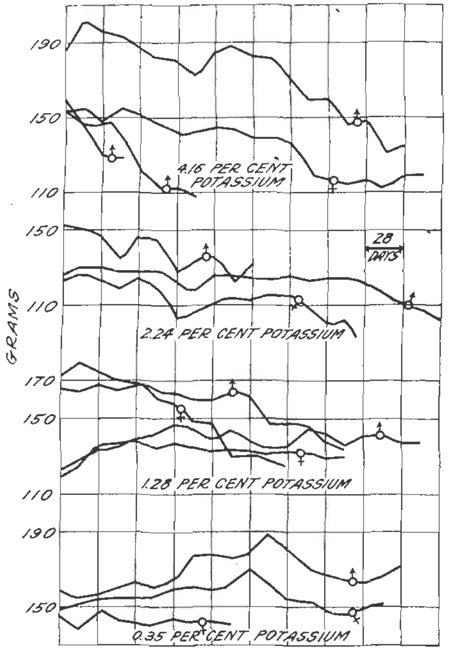

FIG. 1.—Growth curves, showing that rations containing added potassium do not maintain partially grown rats

is quite untenable and then feel that their results show the rapidity with which the animal body can adjust itself to sudden changes in the diet. The same authors (*9*) believe that their results indicate *that their animals assimilated more of the chlorine than of the sodium when salt was added to the ration. Mitchell and Carman (6) im-*proved growth by adding sodium chloride to a corn ration.

EXPERIMENTAL DATA

POTASSIUM

The basal ration used in the experiments on potassium was the same as that described by Olson and St. John (7) and consisted of

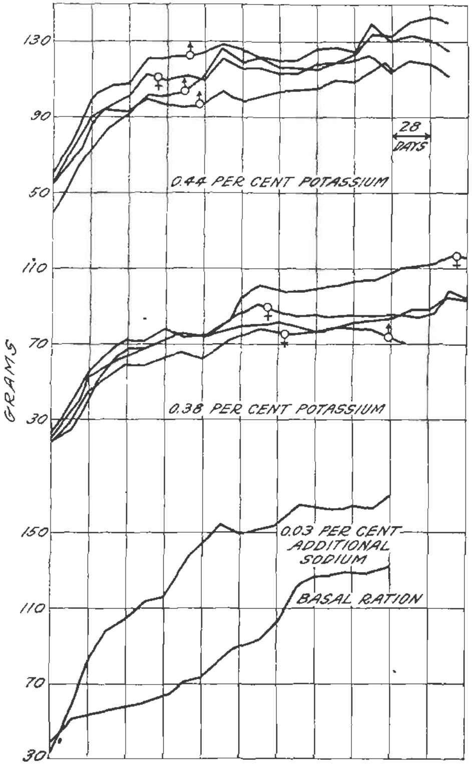

FIG. 2.—Curves showing the effect of potassium on the growth of young rats

100 gm. of wheat, 10 gm. of gluten, 5 gm. of prepared butterfat, and 0.5 gm. of calcium chloride. This ration contained 0.32 per cent

potassium. Different amounts of potassium bicarbonate were added to the basal ration to form rations containing from 0.35 to 4.16 per cent of potassium.

Stock rats of fair size in good condition and growing rapidly were placed on these rations to determine the effect of the added potassium upon maintenance. The growth curves for the animals receiving the minimum and the largest amounts of added potassium are shown in Figure 1. The ration containing the minimum of potassium appears to be about a maintenance ration for animals of this age. Reference to the growth curve for the basal ration shown in Figure 2, copied from the paper by Olson and St. John (7), indicates that the basal ration carried the animals slowly to about 130 gm. and then main-

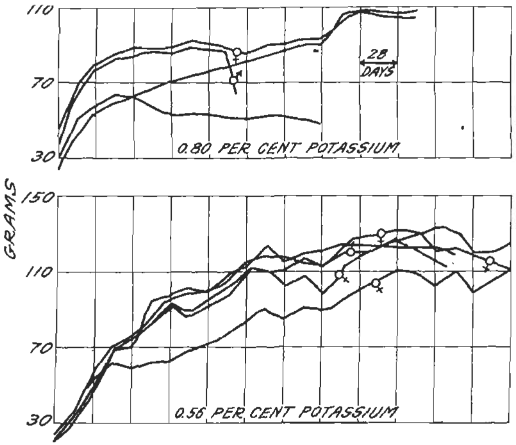

FIG. 3.—Curves showing the effect of potassium on the growth of young rats

tained them, while with sodium added growth was very much improved. On rations containing amounts of potassium from 1.28 to 4.16 per cent the animals declined in weight. The average amount of food consumed per animal per week was 69.5 gm. on the ration containing the smallest amount of potassium and 59.0, 64.4, and 75.7 gm. on the three containing the larger amounts of potassium. The ratio of potassium to sodium was, respectively, 1.52:1, 5.56:1, 9.73:1, 18.1:1 in these four rations.

Figures 2 and 3 present the growth curves for rats on four rations containing from 0.38 to 0.80 per cent of potassium. Variation of this element in the ration when added in the form of potassium bicarbonate seems to have comparatively little effect on the growth of young rats. Increasing the amount did not effect the improvement

in growth which was effected by the addition of increasing amounts of sodium in the form of sodium bicarbonate as shown by Olson and St. John (7). The ratio of potassium to sodium in these rations varied from 1.65:1 to 3.47:1. The amount of feed consumed per week by these animals increased with increasing amounts of potassium from about 32 gm. to about 40 gm. per rat. There was no reproduction among the rats on any of these rations and no pregnancies were noted.

CHLORINE

The same basal ration was used for the study of chlorine as for the study of sodium and potassium. To the wheat, gluten, and butterfat were added various combinations of calcium chloride, calcium carbonate, sodium bicarbonate, and sodium chloride, the proportions being arranged to keep the percentage of calcium constant and the sodium constant (except in the ration containing the largest amount of chlorine), while the chlorine varied from the 0.05 per cent in the basal ration to a maximum of 1.14 per cent. The mineral additions to the rations are shown in Table 1.

TABLE 1.—*Mineral additions made to vary the chlorine content of the ration*

Chemical added	Added to basal ration for —					
	Ration 1	Ration 2	Ration 3	Ration 4	Ration 5	Ration 6
	Gm.	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*
Concentrated HCl (c. c.)						2.00
$CaCl_2$		0.125	0.250	0.500	0.50	.50
$CaCO_3$	0.45	.338	.225			
$NaHCO_3$	.572	.572	.572	.572		
NaCl					3.18	.40
Cl (per cent)	.05	.12	.19	.32	1.14	1.14

Figures 4 and 5 present the growth curves for rats on rations containing four different amounts of chlorine. The growth response on all of the rations appears to be about the same irrespective of the amount of chlorine present. The ration used by Olson and St. John (7) contained an intermediate amount of chlorine (ration D, 0.32 per cent Cl) and the response was here also about the same as in this work when an equal amount of sodium was used. The basal ration with only 0.05 per cent of chlorine produced as good growth as where large amounts of chlorine were used. The maximum amount of chlorine used did not appear to have a detrimental effect on growth, as proved to be the case when large amounts of sodium and potassium in the form of bicarbonate were employed. It will be noted from Figures 4 and 5 that reproduction was secured in several cases. The amount of feed consumed per rat per week decreased to some extent as the amount of chlorine in the ration increased.

It is interesting to note that in the ration containing the largest amount of chlorine a part of this element was added in the form of sodium chloride, so that the percentage of sodium was increased in this one ration and was present to the extent of 1.05 per cent, which amount had proved somewhat detrimental when added in the form of sodium bicarbonate. This suggests that the form in which an element occurs in a diet may influence its effect on growth. This point

should be studied further. Another ration in which hydrochloric acid was used to bring the chlorine content up to 1.14 per cent gave very good growth and very satisfactory reproduction.

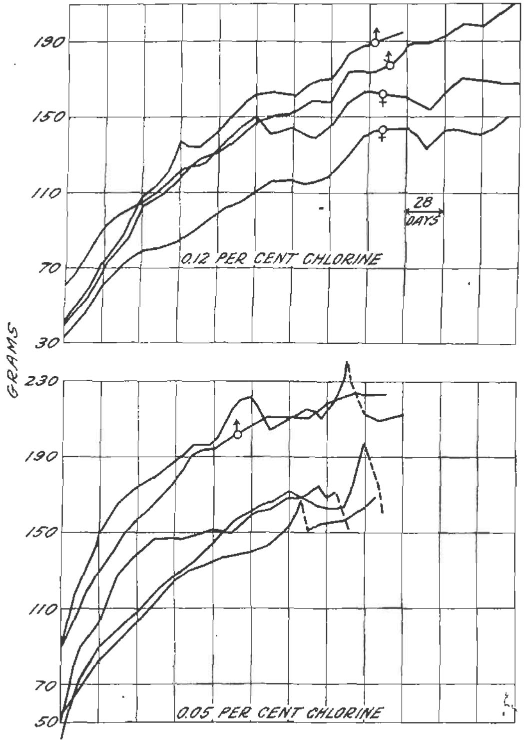

FIG. 4.—Curves showing effect of chlorine on the growth of young rats

DISCUSSION

The value of the ratio of potassium to sodium has been emphasized by other writers. In the work of the present writer this ratio has been varied from 0.12:1 to 18.1:1. With the wheat ration

described above, the best results were obtained when this ratio was 0.6:1, the percentage of potassium and sodium being 0.32 and 0.53, respectively. With a ration of purified food materials containing casein, starch, lard, yeast, cod-liver oil, and an inorganic salt mixture, the writer (*12*) obtained the best results when the ratio was 1.76:1; a ratio of 2.76:1 gave very much poorer results. In all of this work the

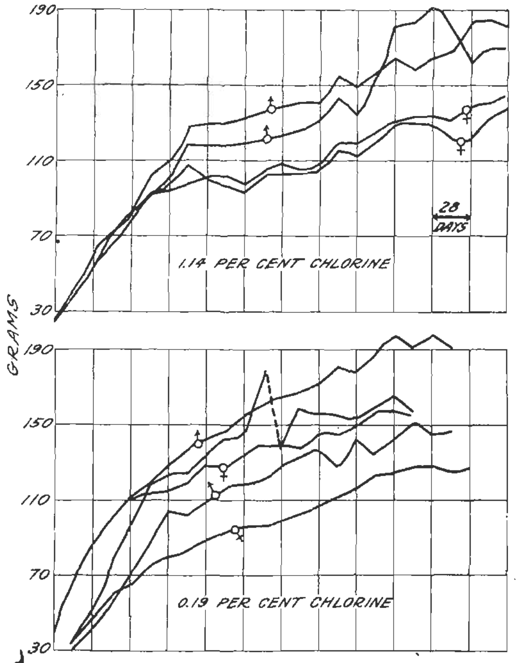

FIG. 5.—Curves showing the effect of chlorine on the growth of young rats

best results were obtained when sodium was present to the extent of about 0.5 per cent of the ration. From this and all of the work with which the author is acquainted it appears that there is not yet sufficient evidence to show that the ratio of potassium to sodium is in itself more important than the actual quantity of these elements in the ration. On the contrary, the evidence seems to support the view that the quantity present is the important factor and that the ratio

may be comparatively wide. What the ratio will be when the optimum for both of these elements has been finally determined is still a question.

The addition of potassium in the form of potassium bicarbonate to a ration based on wheat had very little beneficial effect on the growth of young rats. Neither was the addition of this element to the ration accompanied by reproduction. The results are in contrast to those obtained with sodium which had previously been found beneficial to growth and reproduction. This work emphasizes the view that sodium and potassium are not interchangeable, and it is apparent that potassium will not replace sodium in the ration used. Potassium when present to the extent of over 1 per cent of the ration proved detrimental to animals which had attained a weight of approximately 150 gm.

Although the experimental animals were not kept on screens, as were those used by the writer in a previous experiment (*12*), this fact would have no bearing on the results obtained in view of the work of Kennedy and Palmer (*2*), who state that it does not seem probable that the feces can add anything to the protein, inorganic salts, or energy content of the ration. They appear to feel confident that the feces do not supplement the ration by adding any one of the recognized food factors, and postulate a new vitamin. Such a vitaminlike substance would undoubtedly not be deficient in the type of ration reported upon here, so that the practice of coprophagy would not have a bearing upon these results.

The amount of feed used per gram of gain by the rats receiving the potassium bicarbonate was found to be somewhat greater than when potassium in this form was not added. Increasing the quantity of potassium in the ration appears to increase the food requirement per unit of gain. But Olson and St. John (*7*) have shown that an increase in the percentage of sodium in this ration effected a decided economy in the use of the feed, since the feed requirement per gram gain was decidedly less where larger proportions of sodium were incorporated in the feed. The effect of the potassium in decreasing the feed economy may be explained by the results of Richards, Godden, and Husband (*10*), who found that the addition of potassium to a ration of cereal grain led to a decreased assimilation of nitrogen, phosphorus, and calcium. The results reported by Olson and St. John (*7*) do not, however, agree with the assumption indorsed by these investigators that any ordinary ration will contain sufficient sodium and potassium, since it was found that sodium very materially improved the ration used.

It has been noted that there was no reproduction among the rats on the potassium rations while reproduction occurred among those on sodium rations, as previously reported. The work of Jacques Loeb and his coworkers may suggest a reason for this variation between sodium and potassium. They found that when the fertilized eggs of *Fundulus heteroclitus* were placed in a solution of either sodium chloride or potassium chloride a poisonous action was exerted by these salts. A solution containing both salts was less poisonous than a solution of either one alone, the relative toxicity varying with a variation in the relative concentration. According to Loeb and Wasteneys (*3*), it requires a much larger concentration of sodium chloride to exert a poisonous action than of potassium chloride.

Bechhold (*1, p. 378*), in discussing the work of other authors along this line, states that the potassium ions are especially poisonous because they change the state of turgescence of the organ colloids. If we assume that potassium and sodium salts have a similar action on the fertilized ovum of the rat, we have an explanation of the fact that reproduction was better among the rats on the rations containing added sodium than among those on rations containing added potassium. The addition of small quantities of potassium to the ration may have raised the concentration sufficiently to be toxic. The comparatively high concentration of potassium in the synthetic ration used by the present writer (*12*) may also have been a factor in the failure to secure reproduction among the rats on those rations.

SUMMARY

The results obtained by varying potassium and chlorine in a ration based upon wheat are reported. When rats weighing approximately 150 gm. were fed this ration with potassium bicarbonate added in quantity sufficient to incorporate more than 1 per cent of potassium the rats lost weight. Young animals receiving rations containing 0.4 to 0.8 per cent of potassium grew at about the same rate as those that received the basal ration. None of the animals receiving rations containing added potassium for any length of time produced young. Comparing these results with those previously reported, it appears that potassium will not replace sodium in a ration of this type.

The addition of chlorine to the wheat ration used did not effect an improvement in growth. Reproduction occurred in some instances among rats on this ration, although there is little indication that it varied with the quantity of chlorine present.

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